

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
23 December 2004 (23.12.2004)

PCT

(10) International Publication Number
WO 2004/110360 A2

(51) International Patent Classification⁷: **A61K**
(21) International Application Number:
PCT/US2004/015662
(22) International Filing Date: 17 May 2004 (17.05.2004)
(25) Filing Language: English
(26) Publication Language: English
(30) Priority Data:
60/471,300 16 May 2003 (16.05.2003) US
60/471,239 16 May 2003 (16.05.2003) US
60/471,240 16 May 2003 (16.05.2003) US
60/474,372 29 May 2003 (29.05.2003) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

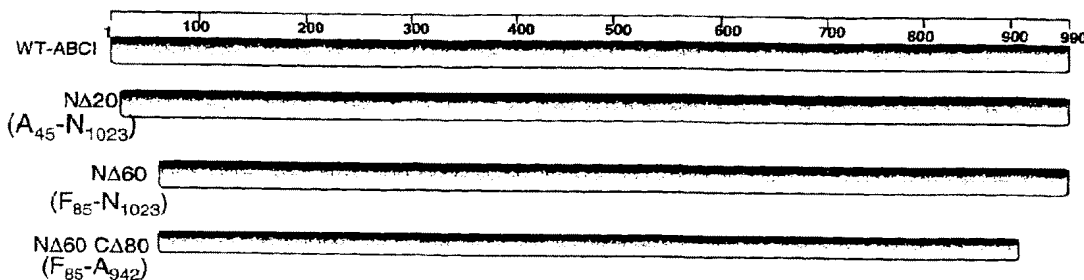
(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: PROTEOGLYCAN DEGRADING MUTANTS FOR TREATMENT OF CNS



(57) Abstract: The present disclosure relates to the preparation and deletion mutants of chondroitinase proteins and their use in methods for promoting the diffusion of therapeutic composition into tissues and their use for neurological functional recovery after central nervous system ("CNS") injury or disease.

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PROTEOGLYCAN DEGRADING MUTANTS FOR TREATMENT OF CNS**CROSS-REFERENCE TO RELATED APPLICATIONS****BACKGROUND AND SUMMARY**

[0002] Chondroitinases are enzymes of bacterial origin that act on chondroitin sulfate, a component of the proteoglycans that are components of the extracellular matrix of a wide variety of tissues such as the central nervous system and for example they can mediate the attachment between the retina and the vitreous body of the human eye. Examples of chondroitinase enzymes are chondroitinase ABC I, **SEQ ID NO: 37**, which is produced by the bacterium *Proteus vulgaris* (*P. vulgaris*), and chondroitinase AC, **SEQ ID NO: 5**, which is produced by *Flavobacterium heparinum*. Chondroitinases ABC I **SEQ ID NO: 37**, and chondroitinase AC **SEQ ID NO: 5**, function by degrading polysaccharide side chains in protein-polysaccharide complexes, without degrading the protein core.

[0003] Yarnagata et al. (J. Biol. Chem. 243:1523-1535, 1968) describe the purification of the chondroitinases like ABC I **SEQ ID NO: 37** from extracts of *P. vulgaris*. This enzyme selectively degrades the glycosaminoglycans chondroitin-4-sulfate, dermatan sulfate, and chondroitin-6-sulfate (also referred to respectively as chondroitin sulfates A, B, and C which are side chains of proteoglycans) at pH 8 at higher rates than it degrades chondroitin or hyaluronic acid. The products of the degradation are high molecular weight unsaturated oligosaccharides and an unsaturated disaccharide. However, chondroitinase ABC I, **SEQ ID NO: 37**, does not act on keratosulfate, heparin or heparitin sulfate.

[0004] Uses of chondroitinases include rapid, specific and non-surgical disruption of the attachment of the vitreous body to the neural retina of the eye, thereby facilitating removal of the vitreous body.

[0005] *P. vulgaris* chondroitinase, for example ABC I **SEQ ID NO: 37** migrates with an apparent molecular mass of about 110 kDa when resolved by SDS-PAGE. The appearance of a doublet in SDS-PAGE resolution of chondroitinase ABC has been reported (Sato et al., Agric. Biol. Chem. 50:4,1057-1059, 1986). However, this doublet represents intact chondroitinase ABC and a 90 kDa degradation product. Commercial chondroitinase ABC protein preparations contain variable amounts of this 90 kDa degradation product and an additional 18 kDa degradation product also derived from chondroitinase ABC I, **SEQ ID NO: 37**.

[0006] Chondroitinase ABC II, **SEQ ID NO: 27**, has also been isolated and purified from *P. vulgaris*, Chondroitinase ABC II, **SEQ ID NO: 27**, is a polypeptide of 990 amino acids with an apparent molecular mass by SDS-PAGE of about 112 kDa. Its molecular mass as determined by electrospray and laser desorption mass spectrometry is about 111,772 daltons. Chondroitinase ABC II, **SEQ ID NO: 27**, has an isoelectric point of 8.4-8.45. Its enzymatic activity is distinct from, but complementary to, that of chondroitinase ABC I **SEQ**

ID NO: 37. Chondroitinase ABC I, **SEQ ID NO: 37**, endolytically cleaves proteoglycans to produce end-product disaccharides, as well as at least two other products which are thought to be tetrasaccharides, Chondroitinase ABC II, **SEQ ID NO: 27**, digests at least one of these tetrasaccharide products from the chondroitinase ABC I (**SEQ ID NO: 37**) digestion of proteoglycan.

[0007] After a injury in the adult mammalian central nervous system (CNS), the inability of axons to regenerate may lead to permanent paralysis. An injury-caused lesion will develop glial scarring, which contains extracellular matrix molecules including chondroitin sulfate proteoglycans (CSPGs). CSPGs inhibit nerve tissue growth *in vitro*, and nerve tissue regeneration fails at CSPGs rich regions *in vivo*.

[0008] A number of molecules, and specified regions of them, have been implicated in the ability to support the sprouting of neurites from a neuronal cell, a process also referred to as neurite outgrowth. The term neurite refers to both axon and dendrite structures. This process of spouting neurites is essential in neural development and regeneration, especially after physical injury or disease has damaged neuronal cells. Neurites elongate profusely during development both in the central and peripheral nervous systems of all animal species. This phenomenon pertains to both axons and dendrites. However, neurite regrowth in the CNS decreases as the animal's age increases.

[0009] Chondroitinase enzymes have shown efficacy in improving functional outcomes in several *in vivo* models of spinal cord injury. Recombinantly produced chondroitinases AC (**SEQ ID NO: 5**) and chondroitinase B (**SEQ ID NO: 12**) polypeptides have shown efficacy *in vitro* by overcoming the barrier of an inhibitory substrate border, such as aggrecan, resulting in neurite extension for rat cortical neurons.

[0010] The inventors have discovered through a deletion analysis based on the available crystal structures, mutant polypeptides capable of degrading chondroitin sulfate

proteoglycans (CSPGs). The cleavage activity of all these mutants have been screened *in vitro* by zymographic assay using aggrecan as a substrate. A truncated polypeptide of chondroitinase AC (n Δ 50-c Δ 275), (SEQ ID NO: 11), lacking 50 and 275 amino acids from the amino and carboxy termini respectively and having a molecular weight of 38 kDa compared to 75kDa of the full length protein, was found to be the minimal size that retained activity as tested by a zymographic assay. The deletion mutant of chondroitinase B (n Δ 120-c Δ 120), (SEQ ID NO: 17), lacking 120 amino acids from each of the amino and carboxy termini and having a molecular weight of 26 kDa compared to 52kDa of the full length protein, was shown to retain activity as well in a zymographic assay. Reduction in the size and complexity of the molecule may facilitate diffusion to the site of action and potentially reduce immunogenicity for prolonged therapeutic use. These smaller chondroitinases could be potential therapeutics for spinal cord injury.

[0011] The present disclosure relates to mutants of chondroitinase genes, polypeptides and proteins derived therefrom, and their use in methods for promoting neurological functional recovery after central nervous system ("CNS") injury or disease. The mutant genes, polypeptides and proteins derived from them preferably include deletion, substitution, or a combination of these from the structural units the mature gene or polypeptide; more preferably the mutant genes or polypeptides are deletion mutants of the mature gene or polypeptide. These mutant genes or polypeptides, preferably biologically active, may be used in various pharmaceutical compositions.

[0012] Polypeptide mutants of chondroitinases, for example chondroitinase ABC Type I, SEQ ID NO: 1 or 37, Chondroitinase ABC Type II, SEQ ID NO: 27, Chondroitinase AC, SEQ ID NO: 5, and Chondroitinase B, SEQ ID NO: 12, are provided. Other mammalian enzymes mutants with chondroitinase-like activity may independently include such enzymes as hyaluronidase 1, SEQ ID NO: 30, hyaluronidase 2, SEQ ID NO:

31, hyaluronidase 3, **SEQ ID NO: 32**, hyaluronidase 4, **SEQ ID NO: 33**, and optionally PH-20, **SEQ ID NO: 34**. These deletion or substitution mutant may be used alone or in combination with chondroitinases or their deletion or substitution mutants as therapeutic compositions and mixtures. Further provided is the use of these mutants, and preferably the chondroitinase deletion or substitution mutants to promote neurological functional recovery in mammals following injury to the CNS, including but not limited to contusion injury.

[0013] One embodiment of the present invention are isolated nucleic acid molecules consisting of, and preferably comprising, a nucleotide sequence encoding the amino acid sequence of polypeptides that are deletion and or substitution mutants of proteoglycan degrading molecules. Independently, nucleic acid molecules of the present invention may encode for mutant proteoglycan degrading polypeptides of chondroitinase, for example chondroitinase ABC Type I, **SEQ ID NO: 1 or 37**, Chondroitinase ABC Type II, **SEQ ID NO: 27**, Chondroitinase AC, **SEQ ID NO: 5**, and Chondroitinase B, **SEQ ID NO: 12**, hyaluronidase 1, **SEQ ID NO: 30**, hyaluronidase 2, **SEQ ID NO: 31**, hyaluronidase 3, **SEQ ID NO: 32**, hyaluronidase 4, **SEQ ID NO: 33**, or optionally PH-20, **SEQ ID NO: 34** and combinations of these. Preferably the nucleic acids encode for chondroitinase deletion and or substitution mutants, most preferably the nucleic acids encode for chondroitinase ABC type I or II polypeptides. The invention is also directed to nucleic acid molecules consisting of, and preferably comprising, a nucleotide sequence complementary to the above-described nucleic acid sequences. Also provided for are nucleic acid molecules at least 80%, preferably 85% or 90%, still more preferably 95%, 96%, 97%, 98%, or 99% identical to any of the above-described nucleic acid molecules. Also provided for are nucleic acid molecules which hybridize under stringent conditions to any of the above-described nucleic acid molecules. The present invention also provides for recombinant vectors comprising these nucleic acid molecules, and host cells transformed with such vectors.

[0014] Also provided are isolated polypeptides consisting of, and preferably comprising, the amino acid sequence of deletion and or substitution mutants of proteoglycan degrading polypeptides. Independently, proteoglycan degrading polypeptides can include chondroitinases, for example ABC Type I, **SEQ ID NO: 1 or 37**, Chondroitinase ABC Type II, **SEQ ID NO: 27**, Chondroitinase AC, **SEQ ID NO: 5**, and Chondroitinase B, **SEQ ID NO: 12**, hyaluronidase 1, **SEQ ID NO: 30**, hyaluronidase 2, **SEQ ID NO: 31**, hyaluronidase 3, **SEQ ID NO: 32**, hyaluronidase 4, **SEQ ID NO: 33**, optionally PH-20, **SEQ ID NO: 34**. Preferably the polypeptides are deletion mutants of chondroitinases. Pharmaceutical compositions may be prepared from the mutant proteoglycan degrading molecules such as these chondroitinases and or hyaluronidases; the composition may include one or more of the deletion and substitution mutants from different proteoglycan degrading polypeptides.

[0015] In one aspect of the invention, biologically active proteoglycan degrading polypeptide are provided having a deletion or substitution of at least one amino acid. The mutant proteoglycan degrading polypeptides include those having the minimal size yet retain a degree of activity as determined by the enzyme assays described in the specification. Preferred deletion or substitution mutants of the proteoglycan degrading molecule are those which degrade chondroitin and have one or more amino acid deletions from the N-terminus, about 1 to at least about 120 amino acids and/or the C-terminus, about 1 to at least about 275 amino acids, more preferably the deletions are from a chondroitinase or a substituted chondroitinase, and even more preferably chondroitinase ABC I or II or a substituted chondroitinase ABC I or II.

[0016] One aspect of this invention are deletion and or substitution mutants of proteoglycan degrading polypeptides, preferably deletion mutants of chondroitinase polypeptides, that promote neurite regeneration and or plasticity in the CNS and or promote or inhibit the diffusion of therapeutic molecules into tissues by degradation of proteoglycans.

[0017] The mutant proteoglycan degrading polypeptides, preferably deletion and or substitution mutants of chondroitinases, may promote neurite regeneration in the CNS and or promote or inhibit the diffusion of therapeutic molecules into tissues by degradation of proteoglycans and can be obtained through expression of suitably modified DNA sequences. Thus, the present invention also provides suitable expression vectors and host cells compatible with them.

[0018] In yet other aspects, the invention comprises pharmaceutical compositions that include biologically active polypeptide of deletion and or substitution mutants of proteoglycan degrading molecules, and preferably deletion or substitution mutants of chondroitn degrading polypeptides as described above, in combination with a pharmaceutically acceptable carrier.

[0019] The deletion mutants and or substitution mutants of the proteoglycan degrading polypeptides of the present invention may be used to promote the regeneration of neurites in nerve tissue. These mutants might also be useful in the treatment of other CNS disorders in which plasticity, regeneration, or both might be beneficial. For example CNS injuries and disorders may include but not limited to contusion injury, traumatic brain injury, stroke, multiple sclerosis, brachial plexus injury, ambliopia. Because of their proteoglycan degrading properties, they may be used to promote the delivery of theraprutic compositions and diagnostics to tissues and cells that are normally impermeable to them. Alternatively, they may be used to inhibit penetration of therapeutic compositions, diagnositics or cells to tissues that use part of the extracellular matrix to enter tissues. Because of their smaller size compared to the full length enzyme, the deletion and or substitution mutants are easier to make and easier to deliver to target cells and tissues. These and other even smaller deletion or substitution mutants of proteoglycan degrading molecules could be used as potential

therapeutics with lesser immunogenicity and similar or higher tissue penetration ability for the treatment of CNS injury.

[0020] The deletion mutants may offer significant advantages over the full length proteins in the therapeutic development process. The tissue penetration of the enzymes may be significantly effected by the protein size. The effect of protein size on tissue penetration is difficult to predict, but dependent on size and charge. The rate of penetration depends on tissue composition, charge interactions and hydration effects. Having active enzymes of widely ranging size may allow selection of an enzyme based on optimal tissue penetration properties, perhaps maximizing effective concentrations or limiting peripheral exposure to the enzyme.

[0021] The immune response of a mammal to a bacterial protein may or may not limit the ability to use the protein or polypeptide as a therapeutic. The generation of antibodies to the protein can restrict repeated exposures, as well as potentially inactivate the protein therapeutic making it ineffective. The smaller mutant proteoglycan degrading enzymes, preferably mutant chondroitinase enzymes, may limit the antigenic sites, limit an immune response or at least simplify the process of engineering an enzyme with reduced immunogenicity.

[0022] The release rate of proteins from matrices often used in sustained release formulations can be dependent upon size and cross-linking. The effective release rate of deletion mutants of proteoglycan degrading polypeptide from the matrix can be engineered through the manipulation of the size of the enzyme. Having a repertoire of chondroitinase enzymes of various size and charge will give a significant advantage for the development of a sustained release formulations.

A BRIEF DESCRIPTION OF THE FIGURES

[0023] FIG. 1(A) shows Anti-His-tag Western Blot (top) and zymogram (bottom) demonstrating chondroitinase B deletion N Δ 120 C Δ 120 mutant (SEQ ID NO: 17) expression activity; FIG. 1(B) shows Anti-His-tag Western Blot (top) and zymogram (bottom) demonstrating chondroitinase AC deletion N Δ 50 C Δ 275 mutant (SEQ ID NO: 11) expression activity;

[0024] FIG. 2 shows illustrates the relative substrate degrading activity of various deletion mutant polypeptides of Chondroitinase AC (SEQ ID NO: 6-11) relative to the full length Chondroitinase AC SEQ ID NO: 5 ;

[0025] FIG. 3(A) shows a schematic of deletion mutant polypeptides of chondroitinase AC (SEQ ID NO: 6-11); FIG. 3(B) shows confirmation of chondroitinase AC deletion mutants by Western blotting;

[0026] FIG. 4. shows confirmation of protein expression and catalytic activity of Chondroitinase AC deletion mutants (SEQ ID NO: 6-11) by (A) Western Blotting and (B) zymography;

[0027] FIG. 5 shows a schematic of deletion mutant polypeptides (SEQ ID NO: 13-17) of chondroitinase B (SEQ ID NO: 12);

[0028] FIG. 6 shows confirmation of protein expression and catalytic activity of Chondroitinase B and deletion mutants (SEQ ID NO: 12-17) by (A) Western Blotting and (B) zymography;

[0029] FIG. 7 shows a schematic of Chondroitinase ABC I deletion mutant polypeptides (SEQ ID NO: 2-4) of Chondroitinase ABC I SEQ ID NO: 1;

DETAILED DESCRIPTION

[0030] Before the present compositions and methods are described, it is to be understood that this invention is not limited to the particular molecules, compositions, methodologies or protocols described, as these may vary. It is also to be understood that the terminology used in the description is for the purpose of describing the particular versions or embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

[0031] It must also be noted that as used herein and in the appended claims, the singular forms “a”, “an”, and “the” include plural references unless the context clearly dictates otherwise. Thus, for example, reference to a “cell” is a reference to one or more cells and equivalents thereof known to those skilled in the art, and so forth. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the present invention, the preferred methods, devices, and materials are now described. All publications mentioned herein are incorporated by reference. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

[0032] “Optional” or “optionally” means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where the event occurs or material is present and instances where the event does not occur or where the material is not present.

[0033] One aspect of the present disclosure relates to a series of deletion and or substitution mutants of chonchoitinase genes that can be used to generate deletion mutant enzymes with substantially lower molecular weight, but modified, and preferably equivalent

or superior proteoglycan degrading catalytic activity compared to the wild type enzymes. The deletion and or substitution mutants can be generated by polymerase chain reaction. The resulting mutants are expressed and then enzymatic activity of the mutant polypeptide can be confirmed by using zymography.

[0034] The mutants of the proteoglycan degrading molecules can be used to treat mammalian CNS injuries, typically caused by trauma or disease. In particular, a deletion mutant of a proteoglycan degrading polypeptide molecule like chondroitinase, for example ABC Type I, (SEQ ID NO: 1 or 37), Chondroitinase ABC Type II, (SEQ ID NO: 27), Chondroitinase AC, (SEQ ID NO: 5), and Chondroitinase B, (SEQ ID NO: 12), or mammalian enzymes with chondroitinase-like activity such as hyaluronidase 1, (SEQ ID NO: 30), hyaluronidase 2, (SEQ ID NO: 31), hyaluronidase 3, (SEQ ID NO: 32), hyaluronidase 4, (SEQ ID NO: 33), and optionally PH-20, (SEQ ID NO: 34), or mixtures of any of these may be used to provide a therapeutic treatment for CNS injuries and disorders which may include but not limited to contusion injury, traumatic brain injury, stroke, multiple sclerosis, brachial plexus injury, ambliopia, spinal cord injuries. Spinal cord injuries includes disease and traumatic injuries, such as the crushing of neurons brought about by an auto accident, fall, contusion, or bullet wound, as well as other injuries. Practice of the present methods can confer clinical benefits to the treated mammal, providing clinically relevant improvements in at least one of the subject's motor coordination functions and sensory perception. Clinically relevant improvements can range from a detectable improvement to a complete restoration of an impaired or lost function of the CNS.

[0035] Mutants of proteoglycan degrading molecules, for example the deletion mutants of Chondroitinase AC (SEQ ID NO: 5), may have their enzyme activity stabilized by the addition of excipients or by lyophilization. Stabilizers may include carbohydrates, amino acids, fatty acids, and surfactants and are known to those skilled in the art. Examples

include carbohydrates such as sucrose, lactose, mannitol, and dextran, proteins such as albumin and protamine, amino acids such as arginine, glycine, and threonine, surfactants such as TWEEN® and PLURONIC®, salts such as calcium chloride and sodium phosphate, and lipids such as fatty acids, phospholipids, and bile salts. The stabilizers may be added to the proteoglycan degrading polypeptide deletion mutants in a ratio of 1:10 to 4:1, carbohydrate to polypeptide, amino acids polypeptide, protein stabilizer to polypeptide, and salts to polypeptide 1:1000 to 1:20; surfactant to polypeptide; and 1:20 to 4:1, lipids to polypeptide. Other stabilizers include high concentrations of ammonium sulfate, sodium acetate or sodium sulfate, based on comparative studies with heparinase activity. The stabilizing agents, preferably the ammonium sulfate or other similar salt, are added to the enzyme in a ratio of 0.1 to 4.0 mg ammonium sulfate/IU enzyme.

[0036] The proteoglycan degrading mutant polypeptides may be formulated as compositions and can be administered topically, locally or systemically to a subject or patient. Preferably the subject is a mammal and even more preferably a human in need of a proteoglycan degrading composition such as one of the chondroitinases. Topical or local administration is can be used for greater control of application. One or more proteoglycan degrading mutant polypeptides, singularly or in combination, can be mixed with an appropriate pharmaceutical carrier prior to administration. Examples of generally used pharmaceutical carriers and additives are conventional diluents, binders, lubricants, coloring agents, disintegrating agents, buffer agents, isotonicizing agents, preservatives, anesthetics and the like. Specifically pharmaceutical carriers that may be used are dextran, serum albumin, gelatin, creatinine, polyethylene glycol, non-ionic surfactants (e.g. polyoxyethylene sorbitan fatty acid esters, polyoxyethylene hardened castor oil, sucrose fatty acid esters, polyoxyethylene polyoxypropylene glycol) and similar compounds.

[0037] Compositions of the present invention having a proteoglycan degrading polypeptide or a nucleic acid for expressing it may also include therapeutical molecules, diagnostics, and agents for promoting neurite growth and regeneration. Examples of diagnostic molecules may include but are not limited to fluorescent probes, radioisotopes, dyes, or magnetic contrast agents. Compounds that facilitate plasticity, neurite growth, and regeneration can include but are not limited to molecules that overcome neurite outgrowth inhibition, or promote nerve growth such as soluble NOGO antagonists like NgR₂₇₋₃₁₁, neural cell adhesion molecules like L1, neurotrophic factors, growth factors, phosphodiesterase inhibitors, and inhibitors of MAG or MOG. Additionally, deletion mutants may be combined with other compounds that promote remyelination such as neuregulins (GGF2) and antibodies that promote remyelination.

[0038] Plasticity of the nervous system refers to any type of functional reorganization. This reorganization occurs with development, learning and memory and brain repair. The structural changes that occur with plasticity may include synapse formation, synapse removal, neurite sprouting and may even include strengthening or weakening existing synapses. Regeneration is generally differentiated from plasticity by the long range growth of axons in disrupted tracts that is characteristic of regeneration.

[0039] The biological activity of the proteoglycan degrading molecules of the present invention may be used to control the degradation rate of proteoglycans in a tissue, and for example be chosen to have a slower degradation activity for sensitive tissues and a higher degradation rate for degrading portions of tissue which are thicker. The activity may be controlled by one or more amino acid substitutions or deletions in the polypeptide or vectors used to express them; the activity may be controlled by the concentration or combination of proteoglycan degrading polypeptides in a composition. The proteoglycan degrading activity may be made to be greater or less than that of the full length polypeptide. For example, it can

be made to be less than that of the full length Chondroitinase AC (**SEQ ID NO: 5**), and can be made to be less than half as active as the full length polypeptide as shown in FIG. 2. Also, as further illustrated in FIG. 2, the proteoglycan degrading activity can be made to be greater than the full length Chondroitinase AC (**SEQ ID NO: 5**), it can be made more active than the full length polypeptide by a factor of 1.5 or more; it can be more active than the full length polypeptide by a factor of 2.5 or more.

[0040] Native or wild-type *P. vulgaris* bacterial strains typically can be used to produce chondroitinases ABC I, (**SEQ ID NO: 1 or 37**), and chondroitinase ABC II, (**SEQ ID NO: 27**), and mutants of these full length polypeptide under ordinary growth conditions. Wild-type strains of *P. vulgaris* can be induced to produce detectable levels of chondroitinase ABCI and its mutants by providing an inducing substrate, such as chondroitin sulfate, as the sole carbon source.

[0041] Mutant nucleic acids can be used for expressing mutant proteoglycan degrading polypeptides. The expressed polypeptides or the mutant nucleic acids can be used to treat mammalian CNS injuries, typically caused by trauma or disease. In particular, a deletion mutant nucleic acid for expressing proteoglycan degrading polypeptide molecule like chondroitinase ABC Type I, may include but are not limited to cloned chondroitinase ABC I, (**SEQ ID NO: 22 or 28**), chondroitinase ABC II, (**SEQ ID NO: 26**), nucleic acids for expressing fusion proteins of deletion mutants TAT-chondroitinase ABC I NΔ60 (**SEQ ID NO: 43**) and mutants of these genes in *E. coli* can be expressed using a heterologous expression system with an artificial inducer. Chondroitinase AC (**SEQ ID NO: 22 or 28**), and chondroitinase B (**SEQ ID NO: 26**), and their mutants may be cloned from *F. heparinum* and expressed in *E. coli*.

[0042] The full length proteoglycan degrading molecules like Chondroitinase AC (**SEQ ID NO: 5**), as well as the deletion and or substitution mutants of the proteoglycan

degrading polypeptides may be cloned in a number of bacterial as well as mammalian expression vectors. Non-limiting of these vectors include pET15b, pET14b, pGEX 6P1, pDNA4HisMax, or pSECTag2b. The deletion mutants and substituted polypeptides of the present invention exhibit the ability to degrade proteoglycans such as chondroitin CS and DS, and have a smaller size and molecular weight than the mature enzyme polypeptides which is expected to facilitate their diffusion into cells, tissues and across membranes. Expression vectors can include the nucleic acid sequence that expresses a mutant proteoglycan degrading polypeptide operably linked to an expression control sequence. Operably linked can refer to a linkage between an expression control sequence and coding sequence, where the linkage permits the expression control sequence to control the expression of the coding sequence.

[0043] The properties of the naturally occurring, substituted and or deletion mutants of the proteoglycan degrading molecules may be altered by introducing a variety of mutations in the protein. Such alterations are suitably introduced using the mutagenesis techniques, for example but not limited to PRC mutagenesis, and the mutated polypeptides molecules suitably synthesized using the expression vectors.

[0044] Mutant proteoglycan degrading polypeptides of the present invention include deletions and or substitutions of amino acids from mature proteoglycan degrading polypeptides. Preferably the deletions or substitutions include any two consecutive or separated amino acids, N or C terminal amino acid deletions or substitutions, and internal amino acid deletions or substitutions in the polypeptide. The deletions and or substitutions can start with any amino acid in the molecule and it is possible to have two separated deletions in the molecule. The deletion or substitution results in mutant proteoglycan degrading polypeptide that are smaller than the mature enzyme and retain proteoglycan degrading ability. Mutant proteoglycan degrading polypeptides can be fused or linked to another polypeptide. Polypeptide is used to unambiguously encompasses amino acid sequences

for mutants of any length which have proteoglycan degrading activity and improve plasticity including those minus the signal sequence that is initially part of polypeptide when it is translated and that is cleaved off by a host-translational modification.

[0045] Mutant nucleic acids of the present invention include deletions and or substitutions of nucleotides from genes which express the mature proteoglycan degrading polypeptides. The deletion and substitution mutations at the DNA level are used to introduce amino acid substitutions and or deletions into the encoded protein. These nucleotide deletions and substitutions can be used to introduce deletions and or substitutions into important conformational or active regions of the polypeptide. A nucleic acid fragment is a nucleic acid having fewer nucleotides than the nucleotide sequence encoding the entire amino acid sequence of a mature proteoglycan degrading polypeptide, yet which preferably encodes a mutant polypeptide which retains some biological activity of the full length protein, e.g., the expressed polypeptide fragment retains the ability to induce degradation of proteoglycans, promote diffusion of therapeutics into cells and tissue, or promote regeneration of neurites. Genes encoding either N or C terminal mutants of proteoglycan degrading polypeptide domains linked to other polypeptides can also be used in constructs for expression of fusion proteins linked to mutant proteoglycan degrading polypeptides.

[0046] The deletion and or substitution mutant proteoglycan degrading polypeptides of the present invention may also include derivatives of these polypeptides which have been chemically or enzymatically modified, but which retain their biological activity to degrade proteoglycans. The proteoglycan degrading activity of these mutants may be controlled depending upon the deletion and or substitution made to the polypeptide or the nucleic acid used to express the polypeptide. Variants, fragments, or analogs of the mature proteoglycan degrading polypeptides or nucleic acids and vectors used to express them include mutant polypeptides and nucleic acids having a sequence which differs from the

mature polypeptide or nucleic acid sequence by one or more deletions, substitutions, or a combination of both such that the mutant proteoglycan degrading polypeptides retain their biological activity and can degrade proteoglycans, and preferably degrade chondroitin sulfate proteoglycans.

[0047] Due to the degeneracy of the genetic code, one of ordinary skill in the art will recognize that a large number of the nucleic acid molecules having a sequence at at least 80%, preferably 85% or 90%, still more preferably 95%, 96%, 97%, 98%, or 99% identical to a nucleic acid sequence encoding for a mutant proteoglycan degrading molecule will encode a mutant polypeptide having proteoglycan degrading activity and preferably chondroitin degrading ability. It will be further recognized that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a mutant polypeptide having proteoglycan degrading activity. This is because amino acid substitutions that are either less likely or not likely to significantly effect polypeptide activity (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid) to degrade proteoglycans and preferably to degrade chondroitin.

[0048] Variants included in the invention may contain individual substitutions, deletions or additions to the nucleic acid or polypeptide sequences. Such changes will alter, add or delete a single amino acid or a small percentage of amino acids in the encoded sequence. Variants are referred to as "conservatively modified variants" where the alteration results in the substitution of an amino acid with a chemically similar amino acid.

[0049] The discovery that the proteoglycan degrading activity of the deletion and substitution mutant polypeptides of the present invention can be controlled to be less, about the same, or greater than the full length proteoglycan degrading molecule has another potential advantage. A pharmaceutical composition containing the proteoglycan degrading molecules may be administered parenterally, intravenously or subcutaneously. The use of a

hydrogel composed of biodegradable polymer enclosing the polypeptide and continuously releasing the polypeptide is limited by the amount of polypeptide that can be enclosed in the hydrogel. Using a deletion mutant of the polypeptide with higher specific activity implies that, on a molar basis, more of the active substance can be enclosed in the same volume, thereby increasing the time between successive administrations or possibly avoiding repeated administrations.

[0050] Purification of the polypeptide obtained after expression is dependent on the host cell and the expression construct used. Generally, the purification of proteoglycan deletion or substitution mutants can be performed in the same way as the purification of native full length polypeptides including the use of histidine-tags.

[0051] The deletion or substitution mutant proteoglycan degrading polypeptides and proteins are administered in an amount effective to degrade CSPGs. The polypeptides may be used to aid the diffusion of therapeutic and diagnostic compositions to tissues and and can be used to promote the recovery of neurological function and neurite outgrowth. Once the mutant proteoglycan degrading proteins or polypeptides in the compositions have been purified to the extent desired, they may be suspended or diluted in an appropriate physiological carrier or excipient for SCI treatment or for screening assays of compositions promoting neurite growth *in vitro* on suitable substrates like aggrecan. In models of SCI, effective intrathecal doses of chondroitinases in rats have been about 0.06 units on alternate days for 14 days. A dose for a 70 kilogram human may be about 17 Units. At about 100 Units / milligram, this would equal about 170 micrograms. Doses of up to 20 Units appear safe in mammalian subjects like rats. Compositions may include a proteoglycan degrading mutant polypeptide, preferably mutant chondroitinase polypeptides, and more preferably still deletion mutant chondroitinase polypeptides. These compositions may also include other proteoglycan degrading molecules and deletion and or substitution mutants of them,

molecules which block the action of neurite growth inhibitors, molecules which promote neurite or axon adhesion, diagnostic, therapeutic, or the proteoglycan degrading molecule mutant as part of a fusion protein. The mixture or fusion protein may be added to a carrier or pharmaceutically acceptable excipient can be injected, generally at concentrations in the range of 1 ug to 500 mg/kg of subject. Administering the agent can be by bolus injection, intravenous delivery, continuous infusion, sustained release from implants, or sustained release pharmaceuticals. Administration by injection, can be intramuscularly, peritoneally, subcutaneously, intravenously, intrathecally. Oral administration may include tablets or capsules, preferably the oral dosage is a sustained release formulation for once or twice daily administration. Percutaneous administration can be once per day, and is preferably less than once per day administration. Administration to the human patient or other mammalian subject may be continued until a measurable improvement in autonomic or motor function in the patient is achieved.

[0052] The mutant proteoglycan degrading polypeptides or fusion polypeptides that include them may also be expressed or secreted by genetically modified cells. The expressed deletion or substitution proteoglycan degrading polypeptide or fusion polypeptides may be harvested and purified for a therapeutic composition, or the genetically modified cells can be implanted, either free or in a capsule, at or near the site of CNS injury or a tissue into which the controlled diffusion of therapeutic or diagnostic molecule is desired. Mutant nucleic acids for expressing mutant proteoglycan degrading polypeptides are illustrated by non-limiting examples of chondroitinase ABC I (**SEQ ID NO: 22 and 28**) which encode for substituted chondroitinase ABC I polypeptides and those without leader amino acid sequences; chondroitinase B nucleic acid mutant (**SEQ ID NO: 21**) which encodes for mutant polypeptide NΔ120 CΔ120 of chondroitinase B (**SEQ ID NO: 21**); and chondroitinase AC nucleic acid mutant (**SEQ ID NO: 19**) which encodes for mutant

polypeptide NΔ50 CΔ275 of chondroitinase AC (**SEQ ID NO: 11**). A non-limiting example of a fusion nucleic acid includes a TAT-deletion mutant chondroitinase ABCI fusion DNA construct (**SEQ ID NO: 41**). Another example would be a nucleic acid for TAT-chondroitinase ABCI-NΔ60 (**SEQ ID NO: 43**) for the expressed polypeptide (**SEQ ID NO: 44**).

[0053] Once the mutant proteoglycan degrading polypeptide are administered to cells or a tissue with CSPGs, degradation of CSPGs removes the inhibitory molecules that block neurite outgrowth, and allow the regeneration of neurites into the affected area. The removal of CSPG also promotes plasticity in the CNS. For example, the full length polypeptides of chondroitinase AC (**SEQ ID NO: 5**), and chondroitinase B, (**SEQ ID NO: 12**), degrade CS and DS, respectively, resulting in unsaturated sulfated disaccharides. Chondroitinase AC (**SEQ ID NO: 5**), cleaves CS at 1, 4 glycosidic linkages between N-acetylgalactosamine and glucuronic acid in the polysaccharide backbone of CS. Cleavage occurs through beta-elimination in a random endolytic action pattern. Chondroitinase B (**SEQ ID NO: 12**) cleaves the 1, 4 galactosamine iduronic acid linkage in the polysaccharide backbone of DS. The cleavage of both CS and DS occurs through a beta-elimination process which differentiates these enzymatic mechanisms from mammalian GAG degrading enzymes. Chondroitinase ABC I (**SEQ ID NO: 1**), chondroitinase ABC II (**SEQ ID NO: 27**), are exo and endo lyases that cleave both CS and DS. The removal of CS and DS from a glial scar permits the regeneration of neurite outgrowths into the injured area and promotes plasticity. For example, the proteoglycan degrading molecules illustrated in FIG. 2, Chondroitinase AC (**SEQ ID NO: 5**) and various mutant Chondroitinase AC (**SEQ ID NO: 6-11**) degrade a model proteoglycan substrate at by various amounts. Similar results are shown by *in vitro* zymograph for chondroitinase B (**SEQ ID NO: 12**) and illustrative mutants (**SEQ ID NO: 13-17**) in FIG. 6. It is reasonable to expect that since a proteoglycan

degrading molecule like Chondroitinase ABC I (SEQ ID NO: 1) improves functional recovery in rats with contusive spinal cord injury and also facilitates the diffusion of model compounds into brain tissue, that mutant proteoglycan degrading polypeptides and compositions containing them can also improve functional recovery in mammalian subjects like rats with contusive spinal cord injury and may also facilitates the diffusion of model compounds into brain tissue.

[0054] The regeneration of the nerve cells and restoration of plasticity in the affected CNS area allows the return of motor and sensory function. Clinically relevant improvement will range from a detectable improvement to a complete restoration of an impaired or lost nervous function, varying with the individual patients and injuries. The degree of functional recovery can be demonstrated by improved corticospinal tract conduction, improved tape removal, beam walking, grid walking and paw placement following chondroitinase treatment of a dorsal column lesion. Motor skill improvement as well as autonomic function: bowel, bladder, sensory and sexual function may also be used as measures of function improvement and related to molecular structure and components in the compositions of the present invention.

[0055] A series of polynucleotides that include coding for deletion or substitution mutants of proteoglycan degrading polypeptides may be generated by PCR using the full length cDNAs for the proteoglycans as templates and cloned into an expression vector such as pET15b at the NdeI and BamHI sites for expression in *E. Coli*. After induction of gene expression with isopropyl- β -D-thiogalactopyranoside (IPTG), the bacteria can lysed by sonication with the concomitant extraction of the mutant polypeptide with a surfactant such as Triton X-114/PBS. The majority of recombinant proteoglycan degrading polypeptide may be found in the cytosolic fraction of the bacterial cell lysate and chondroitinase purification protocols can be used to obtain the mutant proteoglycan degrading enzyme with high activity

at high yields. This protocol may include purification by a column having anti-His antibody to selectively bind His-tagged mutant proteoglycan degrading polypeptides and may also include cation-exchange chromatography as a capture step and gel filtration as a polishing step. After these steps, anion exchange membrane filtration, for example Intercept Q, Millipore, can be used for endotoxin and host DNA removal. Following filtration, the proteoglycan degrading mutant polypeptides can be dialyzed into volatile buffer, pH 8.0 and lyophilized to dryness. The final product is expected to be stable at -70°C for long term storage. The pI of the purified basic proteoglycan degrading mutant polypeptide may be determined by IEF-PAGE analysis of the samples from the crude cell lysate.

[0056] A variety of analytical methods can be used to compare the enzymatic activity of the recombinant version the deletion or substitution mutants of proteoglycan degrading polypeptides with those of full length proteoglycan degrading molecules like chondroitinase ABC I (**SEQ ID NO: 37**) or a commercially available form of the enzyme. The methods may also be adapted to evaluate the activity of fusion proteins including a mutant proteoglycan degrading polypeptide portion. Specific activity measurements may be obtained using an accepted spectrophotometric assay that measures the change in absorbance due to the production of reaction products from the degradation of proteoglycans. Size exclusion chromatography can be used to compare the hydrodynamic properties of the mutant enzymes.

[0057] A form of zymography can be used to characterize the mature proteoglycan degrading enzyme and may be adapted for characterization of the mutants proteoglycan degrading polypeptides. Polyacrylamide gels can be polymerized in the presence of aggrecan, a substrate for proteoglycan degrading molecules like chondroitinase ABCI. The mutant proteoglycan degrading polypeptides, enzyme samples, may be resolved on the aggrecan-impregnated gels by electrophoresis in the presence of SDS. The gels can then be

subjected to a renaturation step wherein the SDS can be extracted and the enzymes allowed to refold. The refolded enzyme regains activity then digests aggrecan within the gel and the resulting loss of carbohydrate in that region of the gel that can be visualized by a carbohydrate-specific stain. A similar loss of carbohydrate in the gel would be expected for equally active forms and concentration of the mutant proteoglycan degrading molecules. In the case of recombinant Chondroitinase ABCI, its activity can be visualized as a clear spot in the zymogram. The zymography results are consistent with the spectrophotometric analysis.

[0058] HPLC methods may be used for detecting the four and six sulphated disaccharides ($\Delta 4$ DS and $\Delta 6$ DS, respectively) liberated as a result of mutant proteoglycan degrading polypeptide digestion of CSPG. The two disaccharides can be effectively resolved by anion exchange chromatography. The HPLC assay for the quantitation of $\Delta 4$ DS and $\Delta 6$ DS from chromatograms is expected to yield a linear relationship proportional to the amounts injected into the HPLC. Production of $\Delta 4$ DS and $\Delta 6$ DS from CSPG digestion is directly related to the amount of chondroitinase specific activity as determined by the spectrophotometric assay. This assay may be used as a sensitive and accurate method to independently quantitate $\Delta 4$ DS and $\Delta 6$ DS released by mutant proteoglycan degrading polypeptide digestion of a variety of substrates and may also be used to determine the activity of mutant proteoglycan degrading polypeptides and fusion proteins including them.

[0059] Another functional assay that can be performed to characterize mutant proteoglycan polypeptide activity is where dorsal root ganglion (DRG) neurons are plated on aggrecan or aggrecan treated with a deletion or substitution mutant proteoglycan degrading polypeptide. It is expected that neurons plated on aggrecan will fail to adhere to the plate and extend axons. In contrast, neurons plated on aggrecan treated with a mutant proteoglycan degrading polypeptide in a composition or as part of a fusion polypeptide would be expected to adhere to the surface and extend axons. The extensive axon growth, which is observed for

chondroitinase ABC I (**SEQ ID NO:37**) is believed to be due to the digestion of the carbohydrates on the aggrecan core protein which creates a more permissive substrate for axon growth.

[0060] Various aspects of the invention may be understood with reference to the following non-limiting examples.

EXAMPLE 1

[0061] This prophetic example illustrates the diffusion of molecules into cells and tissue using a deletion or substitution mutant of a proteoglycan degrading polypeptide in a composition.

[0062] A brain from an adult Sprague Dawley rat may be removed from the skull and hemispheres may be soaked in buffer alone or containing about 33U/ml of a mutant proteoglycan degrading polypeptide such as (**SEQ ID NO: 9**) **NΔ50 CA200 AC (T₇₄-T₅₀₀) protein** for 2 hours at 37 °C. Hemispheres can be rinsed and immediately placed in dye such as Eosin Y (Sigma) or a saturated solution of Congo Red (Sigma) in 70% ethanol. Slabs of tissue may be cut and images acquired on a scanner. The penetration of the dyes into the brain tissue may be used as an indication of the proteoglycan degrading activity of a mutant proteoglycan degrading molecule and expectant penetration or diffusion of therapeutic and diagnostic molecules into the same type of tissue.

EXAMPLE 2

[0063] This prophetic example illustrates a Chondroitinase ABC I Assay Protocol which may be modified to measure the activity of a mutant proteoglycan degrading molecule,

for example a Chondroitinase ABCI deletion mutant or a fusion proteins including a deletion and or substitution mutant of a proteoglycan degrading polypeptide.

[0064] The production of reaction products from the catalytic activity of a proteoglycan degrading molecule or fusion protein can be determined by a measurement of the absorbance of the proteoglycan degradation product at a wavelength of 232 nm. A typical reaction mixture consisted of 120 μ l of reaction mixture (40mM Tris, pH 8.0, 40mM NaAcetate, 0.002% casein) combined with a substrate (5 μ l of 50 mM chondroitin C (MW 521), chondroitin 6 SO₄, or dermatan sulfate) and 1.5 μ l of chondroitinase ABCI (**SEQ ID NO:1**) or a mutant of chondroitinase like (**SEQ ID NO:2**). Reaction mixture aliquots of about 120 μ l can be prepared at 30-37°C for 3 min or longer. The product formation is monitored as an increase in absorbance at 232 nm as a function of time at a wavelength of 232 nm using a spectrometer. The reaction may be stopped by addition of 0.1% SDS followed by boiling for 5 minutes. The observed activity may be converted to units (μ moles of product formed per minute) using the molar absorption coefficient for the C4-C5 double bond formed in the reaction ($3800 \text{ cm}^{-1}\text{min}^{-1}$).

[0065] Knowing the molar absorption coefficient for the reaction product, measuring the change in the absorbance of the reaction product at 232 nm reading over time upon addition of a known amount of the Chondroitinase ABCI (**SEQ ID NO:1**) or other other mutant proteoglycan degrading polypeptide to the 120 μ l reaction mixture with 0002% casein and a chondroitin substrate added, the specific activity in $\mu\text{mol}/\text{min}/\text{mg}$ of the mutant proteoglycan degrading polypeptide can be determined. Seikagaku Chondroitinase ABC I has a specific activity under these assay conditions of about $450 \mu\text{mole}/\text{min}/\text{mg}$.

[0066] A proteoglycan degrading molecule like Chondroitinase ABC I (**SEQ ID NO:37**), digests axon growth inhibiting chondroitin present in CNS tissue and improves functional recovery in rats having contusion spinal cord injuries. It is reasonable to expect

that mutants of proteoglycan degrading molecules, such as (SEQ ID NO: 11) **NΔ50 CΔ275 AC (T₇₄-T₄₂₆) polypeptide** that show proteoglycan degrading activity may also show some regeneration of nerves, stimulate plasticity and be useful for diffusion of agents into tissues. The mode of administration, the timing of administration and the dosage are carried out such that the functional recovery from impairment of the CNS is enhanced by the promotion of neurite outgrowth and plasticity. It is reasonable to expect that once the deletion or substitution mutants of proteoglycan degrading molecules such as (SEQ ID NO: 11) **NΔ50 CΔ275 AC (T₇₄-T₄₂₆) protein** are administered, the degradation of CSPGs can remove the inhibitory molecules in tissue that block drug diffusion, block neurite outgrowth, and promote the regeneration of neurites or other therapeutics into the affected area. The regeneration and plasticity of the nerve cells into the affected CNS area may allow the return of motor and sensory function. Clinically relevant improvements will range from a detectable improvement to a complete restoration of an impaired or lost nervous function, varying with the individual patients and injuries.

EXAMPLE 3

[0067] This example shows that deletion mutants of chondroitinase are biologically active.

[0068] Recombinantly produced chondroitinases AC and B have shown efficacy *in vitro* by overcoming the barrier of an inhibitory substrate border, such as aggrecan and result in neurite extension for rat cortical neurons. To facilitate effective transport of the above enzymes to the injury site, deletion mutants of these chondroitinases were prepared to determine the minimally-sized polypeptides capable of degrading CSPGs. The cleavage activity of all these mutants have been screened *in vitro* by zymographic assay using aggrecan as substrate. A truncated polypeptide of chondroitinase AC (nΔ50-cΔ275) (SEQ

ID NO:11) lacking 50 and 275 amino acids from the amino and carboxy termini respectively having a molecular weight of 38 kDa compared to 75kDa of the full length protein was found to be about the minimal size mutant chondroitinase AC that retains activity as tested by zymography assay FIG. 4(B). However, an even smaller mutant, the deletion mutant of chondroitinase B (nΔ 120-cΔ 120) (**SEQ ID NO:17**) lacking 120 amino acids from each of the amino and carboxy termini, having a molecular weight of 26 kDa compared to 52 kDa of the full length protein has also shown to retain activity as well in zymography assay FIG. 6(B). These and other even smaller deletion mutants could be used as potential therapeutics with lesser immunogenicity and similar or higher tissue penetration ability compared to the mature enzyme and may be used for treatment of spinal cord injury.

[0069] A series of chondroitinase AC and B deletion mutants were generated by PCR using the full-length cDNAs for chondroitinases AC and B as templates and cloned in the pET15b expression vector at the NdeI and BamHI sites. Full length and deletion mutants were constructed with Histidine-tags for ease of detection and purification. Each of these cDNAs was induced by Isopropyl-β-D-Thiogalactopyranoside (IPTG,) and the expression was confirmed by Western blotting using anti-His antibody (Novagen). FIG. 3(A) show various non-limiting deletion mutants schematically, and FIG 3(B) shows confirmation of expression of these chondroitinase AC mutant polypeptides by anti-histidine tag Western blotting. Figures 5 and 6 show the same information for chondroitinase B deletions. Western blots demonstrate proteins of predicted size. Zymographic PAGE of deletion mutants show intense bands of substrate digestion (light) and negative carbohydrate staining.

[0070] Zymography assay. SDS-polyacrylamide gels were poured with aggrecan (85 μg/ml) polymerized into it. Crude extracts of deletion mutants of chondroitinases AC and B were run and renatured at 37 °C overnight. After separation the gel is incubated in 0.2% Cetylpyridinium for 90 minutes at room temperature. The digestion of the

proteoglycans by the chondroitinases is visualized by staining the gel with 0.2% Toluidene Blue in ethanol-H₂O-acetic acid (50:49:1 v/v/v) for 30 minutes and destained with ethanol-H₂O-acetic acid (50:49:1 v/v/v). Following destaining the gel is incubated overnight in a 50 µg/ml solution of Stains-all in 50% ethanol in the dark and destained with H₂O. Appearance of clear bands on the gel shows the digestion of carbohydrates by the chondroitinases of the CSPG leaving the core protein which remains unstained (FIG 4. and FIG. 6).

EXAMPLE 4

[0071] This example describes the linking of a His tag to a mutant proteoglycan degrading polypeptide.

[0072] Deletion mutants of the chondroitinase ABC I enzyme where the mutant is missing a certain number of amino acids from the N-terminal and maintains proteoglycan degrading activity can be generated (**SEQ ID NO:2-4**). These N-terminal deletion maintain a histidine-tag that is attached to the N-terminus; however similarly tagged full length chondroitinase ABC I (**SEQ ID NO:1**) did not maintain the histidine-tag after expression.

[0073] Catalytically active deletion mutants of chondroitinase ABC I can be prepared for example but not limited to deleting 20, and 60 amino acids respectively from the N-terminus of the mature ABC I protein as shown in FIG. 7. A mutant polypeptide with both N and C terminal deletions such as chondroitinase ABC I-NΔ60-CΔ80 (**SEQ ID NO:4**) can also be made.

[0074] These chondroitinase deletion mutants and mutants of other proteoglycan degrading molecules may be used for construction of N-terminal fusion chimeric protein. Assay tests with these fusion polypeptides for chondroitin degradation and may be used to determine the efficacy of mature ABCI versus various deletion mutant in compositions and fusion proteins with respect to the substrate specificity, substrate binding and tissue

penetration. Functional assay that can be performed to characterize the activity of mutant proteoglycan polypeptide or fusion polypeptides including them. In this functional assay, dorsal root ganglion (DRG) neurons can be plated on aggrecan or aggrecan treated with a mutant proteoglycan degrading polypeptide or a fusion polypeptide including the mutant. It is expected that neurons plated on aggrecan will failed to adhere to the plate and extend axons. In contrast, neurons plated on aggrecan treated with a mutant proteoglycan degrading polypeptide or a fusion polypeptide including the mutant in a composition or as part of a fusion polypeptide would be expected to adhere to the surface and extend axons. The extensive axon growth, which is observed for a chondroitinase like chondroitinase ABC I (SEQ ID NO: 1 or 37) treated aggrecan substrate is believed to be due to the digestion of the carbohydrates on the aggrecan core protein which creates a more permissive substrate for axon growth.

EXAMPLE 5

[0075] This phrophetic example describes a mutant of chondroitinase ABC I that has native protein structure, but lacks proteoglycan degrading catalytic activity.

[0076] This mutant may be prepared as a null or a negative control for bioassays and SCI studies. Based on the crystal structure of chondroitinase ABC I a site-specific mutant designated H501a and Y508a (SEQ ID NO: 36) to knock out catalytic activity in the putative active site can be prepared. Such mutants can be tested for inactivation of catalytic activity and SEC to compare to the wild-type enzyme. The null activity mutant can also be used to provide a negative control for the various proteoglycan degrading fusion proteins for use in bioassays and ultimately in SCI animal studies.

EXAMPLE 6

[0077] This example illustrates examples of mutant proteoglycan degrading polypeptides that include both substitution and deletions from polypeptides of the present invention.

[0078] The chondroitinase ABC I sequence (SEQ ID NO: 37) is a published sequence for a mature chondroitinase ABC I peptide and includes the leader sequence. Chondroitinase ABC I sequence (SEQ ID NO: 37) is similar to (SEQ ID NO: 1 or 29), however (SEQ ID NO: 1) does not have the first 25 amino acids of (SEQ ID NO: 37), and amino acids at positions 154 and 195 of (SEQ ID NO: 37) differ from those (substitutions) found in similar positions when (SEQ ID NO: 1) and (SEQ ID NO: 37) are aligned.

[0079] (SEQ ID NO: 38-40) illustrate deletions from either the N or C terminal of the (SEQ ID NO: 37) polypeptide and substitutions relative to (SEQ ID NO: 1). These mutant polypeptides are NΔ20 (SEQ ID NO: 38), NΔ60 (SEQ ID NO: 39) and NΔ60 CΔ80 (SEQ ID NO: 40).

EXAMPLE 7

[0080] This example illustrates non-limiting illustrations of mutant polypeptides of the present invention fused with a membrane transduction polypeptide such as but not limited to a polypeptide portion of a HIV TAT protein. Full sequence listings for the mutants fusion polypeptides are provided in the Sequence listing included in the specification.

[0081] A nucleotide sequence for TAT-chondroitinase ABCI-nΔ20 (SEQ ID NO. 41), a portion of which is illustrated below, shows the TAT sequence nucleotides highlighted by underlining linked to chondroitinase nucleotides.

```

1  ggtc gtaaaaagcg tcgtcaacgt cgtcgtcctc ctcaatgcgc aaaaataac
61 ccattagcag acttctcatc agataaaaac tcaatactaa cgttatctga taaacgtacg

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[0082] The underlined nucleotides in this portion of the nucleic acid sequence denote a TAT sequence attached to the 5' of chondroitinase ABC I- Δ 20 nucleic acid (SEQ ID NO. 47).

[0083] An amino acid sequence for TAT-chondroitinase ABCI- Δ 20 (SEQ ID NO. 42), a portion of which is shown below, illustrates the TAT sequence amino acids highlighted by underlining at the N-terminus of chondroitinase ABCI- Δ 20 (SEQ ID NO. 2).

grkkrrrrppqcaqnnpladfsdknsiltlsdkrsimgnqslwkwwggssflhkkliivptdkeaskawgrsstpvsfwly
nekpdygtidfgelklistseaagfkvkldftgwrvtgvslnndlenremtlnatntssdgtqdsigrslgakvdsirfkapsnvsq
geiy

[0084] A nucleotide sequence for TAT-ABCI- Δ 60 (SEQ ID NO. 43), a portion of which is illustrated below, shows the N-terminal TAT (SEQ ID NO. 49) nucleotides highlighted by underlining.

ggtcgtaaaaagcgtcgtcaacgtcgtcgtcctcctcaatgctttactttacataaaaaactgattgtccccaccgataaagaagcatcta
aagcatggggacgctcatccacccccgtttctcatttggctttacaatgaaaaaccgattgatggttatcttactatcgatttcgg.....

[0085] Amino acid sequence for TAT-ABCI- Δ 60 (SEQ ID NO. 44) a portion of which is shown below, illustrates the TAT sequence (SEQ ID NO. 50) highlighted by underlining at the N-terminus of chondroitinase ABC I- Δ 60 (SEQ ID NO. 3).

grkkrrrrppqcflhkkliivptdkeaskawgrsstpvsfwlynekpdygtidfgelklistseaagfkvkldftgwrvtgvsln
ndlenremtlnatntssdgtqdsigrslgakvdsirfkapsnvsqgeiyidrimfsvddaryqwsdyqvtrlsepeiqf....

[0086] Nucleotide sequence for ABCI-TAT-C (SEQ ID NO. 45), a portion of which is illustrated below, shows the C-terminal TAT sequence nucleotides highlighted by underlining. The stop codon from chondroitinase ABC I (SEQ ID NO. 28) was replaced by the TAT sequence and was placed at the 3' end of the TAT sequence.

...gattaatggcaaatggcaatctgctgataaaaatagtgaagtgaaatatcagggttctggtgataaacactgaactgacgtttacgagtt
actttggtattccacaagaaatcaaactctcgccactccct ggtcgtaaaaagcgtcgtcaacgtcgtcgtcctcctcaatgctag

[0087] Amino acid sequence for ABCI-TAT-C (SEQ ID NO. 46), a portion of which is shown below, illustrates the TAT sequence, highlighted by underlining, linked to the chondroitinase polypeptide at the C-terminus of the mature chondroitinase ABC I (SEQ ID NO. 1).

...aekvnvsrqhqvsaaenknrqptegnffsawidhstrpkdasayeymvfldatpekmgemaqkfrennglyqvlrkdvdvhi
ildklsnvtgyafyqpasiedkwikkvnkpaivmthrqkdtlivsavtpdlmtrqkaatpvtinvtingkwqsadknsevkyq
vsgdnteltfysyfgipqeiklsplpgrkkrqrppqc

EXAMPLE 8

[0088] This example illustrates the sequence of chondroitinase nucleic acid and polypeptides which may be used for deletions or substitutions in mutants of the present invention. In these sequence, discrepancies from published sequences are highlighted in bold text at both the nucleotide level and at the amino acid level. These are illustrative of substitutions in the present invention.

SEQ ID NO: 26 Present invention Chondroitinase ABC II Nucleic acid

```
>_ ABC II mature                                2973 nt vs.
>_ ABC II (present invention)                   2974 nt
scoring matrix: , gap penalties: -12/-2
99.0% identity;                               Global alignment score: 11684
```

	10	20	30	40	50	60
806559	TTACCCACTCTGTCTCATGAAGCTTTCGGCGATATTTATCTTTTTGAAGGTGAATTACCC					
—	TTACCCACTCTGTCTCATGAAGCTTTCGGCGATATTTATCTTTTTGAAGGCGAATTACCC					
	10	20	30	40	50	60
	70	80	90	100	110	120
806559	AATACCCCTTACCACTTCAAATAATAATCAATTATCGCTAAGCAAACAGCATGCTAAAGAT					
—	AATATCCTTACCACTTCAAATAATAATCAATTATCGCTAAGCAAACAGCATGCTAAAGAT					
	70	80	90	100	110	120

33

34

35

GGATGGGATTGGAATAGATATCCAGGTACAACAACTATTTCATCTTCCCTATAACGAACTT
1930 1940 1950 1960 1970 1980

1990 2000 2010 2020 2030 2040
806559 GAAGCAAACTTAATCAATTACCTGCTGCAGGTATTGAAGAAATGTTGCTTTCAACAGAA
GAAGCAAACTTAATCAATTACCTGCTGCAGGTATTGAAGAAATGTTGCTTTCAACAGAA
1990 2000 2010 2020 2030 2040

2050 2060 2070 2080 2090 2100
806559 AGTTACTCTGGTGCAAATACCCTTAATAATAACAGTATGTTTGCCATGAAATTACACGGT
AGTTACTCTGGTGCAAATACCCTTAATAATAACAGTATGTTTGCCATGAAATTACACGGT
2050 2060 2070 2080 2090 2100

2110 2120 2130 2140 2150 2160
806559 CCAAGTAAATATCAACAACAAAGCTTAAGGGCAAATAAATCCTATTTCTTATTGATAAT
CACAGTAAATATCAACAACAAAGCTTAAGGGCAAATAAATCCTATTTCTTATTGATAAT
2110 2120 2130 2140 2150 2160

2170 2180 2190 2200 2210 2220
806559 AGAGTTATTGCTTTAGGCTCAGGTATTGAAAATGATGATAAACAACATACGACCGAAACA
AGAGTTATTGCTTTAGGCTCAGGTATTGAAAATGATGATAAACAACATACGACCGAAACA
2170 2180 2190 2200 2210 2220

2230 2240 2250 2260 2270 2280
806559 AACTATTCCAGTTTGCCGTCCCTAAATTACAGTCAGTGATCATTAATGGCAAAAAGGTA
AACTATTCCAGTTTGCCGTCCCTAAATTACAGTCAGTGATCATTAATGGCAAAAAGGTA
2230 2240 2250 2260 2270 2280

2290 2300 2310 2320 2330 2340
806559 AATCAATTAGATACTCAATTAACCTTTAAATAATGCAGATACATTAATTGATCCTGCCGGC
AATCAATTAGATACTCAATTAACCTTTAAATAATGCAGATACATTAATTGATCCTGCCGGC
2290 2300 2310 2320 2330 2340

2350 2360 2370 2380 2390 2400
806559 AATTTATATAAGCTCACTAAAGGACAACTGTAAAATTTAGTTATCAAAAACAACATTCA
AATTTATATAAGCTCACTAAAGGACAACTGTAAAATTTAGTTATCAAAAACAACATTCA
2350 2360 2370 2380 2390 2400

2410 2420 2430 2440 2450 2460
806559 CTTGATGATAGAAATTCAAACCAACAGAACAATTATTTGCAACAGCTGTTATTTCTCAT
CTTGATGATAGAAATTCAAACCAACAGAACAATTATTTGCAACAGCTGTTATTTCTCAT
2410 2420 2430 2440 2450 2460

2470 2480 2490 2500 2510 2520
806559 GGTAAGGCACCGAGTAATGAAAATTATGAATATGCAATAGCTATCGAAGCACAAAATAAT
GGTAAGGCACCGAGTAATGAAAATTATGAATATGCAATAGCTATCGAAGCACAAAATAAT
2470 2480 2490 2500 2510 2520

2530 2540 2550 2560 2570 2580
806559 AAAGCTCCCGAATACACAGTATTACAACATAATGATCAGCCCCATGCGGTAAAAGATAAA
AAAGCTCCCGAATACACAGTATTACAACATAATGATCAGCCCCATGCGGTAAAAGATAAA

The above discrepancies, bold text, at the nucleotide level resulted in 98.3% identity at the amino acid level and the substituted residues are marked in bold text in the following.

```
>_ ABC (present invention)
>_ ABC (mature)
scoring matrix: , gap penalties: -12/-2
98.3% identity; Global alignment score: 6393
```

BNSDOCID: <WO 2004110360A2 | >

38

SEQ ID NO: 28 Present Invention Chondroitinase ABC I nucleic acid

```

      10          20          30          40          50          60
806559 GCCACCAGCAATCCTGCATTGATCCTAAAAATCTGATGCAGTCAGAAATTACCATTTT
      :
      :
      :
-       GCCACCAGCAATCCTGCATTGATCCTAAAAATCTGATGCAGTCAGAAATTACCATTTT
      10          20          30          40          50          60

      70          80          90         100         110         120
806559 GCACAAAATAACCCATTAGCAGACTTCTCATCAGATAAAAACTCAATACTAACGTTATCT
      :
      :
      :
-       GCACAAAATAACCCATTAGCAGACTTCTCATCAGATAAAAACTCAATACTAACGTTATCT
      70          80          90         100         110         120
```


40

41

42

.....
CAAAGTCATGGTGTGCTCAAATAGTGAGTAATGGCTCGCAGCTTTACAGGGCTATCAG
1930 1940 1950 1960 1970 1980

1990 2000 2010 2020 2030 2040
806559 CAAGAAGGTTGGGATTGGAATAGAATGCCAGGGGCAACCACTATCCACCTTCCTCTTAAA
.....
CAAGAAGGTTGGGATTGGAATAGAATGCAAGGGGCAACCACTATTCACCTTCCTCTTAAA
1990 2000 2010 2020 2030 2040

2050 2060 2070 2080 2090 2100
806559 GACTTAGACAGTCCTAAACCTCATACCTTAATGCAACGTGGAGAGCGTGGATTTAGCGGA
.....
GACTTAGACAGTCCTAAACCTCATACCTTAATGCAACGTGGAGAGCGTGGATTTAGCGGA
2050 2060 2070 2080 2090 2100

2110 2120 2130 2140 2150 2160
806559 ACATCATCCCTTGAAGGTCAATATGGCATGATGGCATTCGATCTTATTTATCCCGCCAAT
.....
ACATCATCCCTTGAAGGTCAATATGGCATGATGGCATTCGATCTTATTTATCCCGCCAAT
2110 2120 2130 2140 2150 2160

2170 2180 2190 2200 2210 2220
806559 CTTGAGCGTTTTGATCCTAATTTCACTGCGAAAAAGAGTGTATTAGCCGCTGATAATCAC
.....
CTTGAGCGTTTTGATCCTAATTTCACTGCGAAAAAGAGTGTATTAGCCGCTGATAATCAC
2170 2180 2190 2200 2210 2220

2230 2240 2250 2260 2270 2280
806559 TTAATTTTTATTGGTAGCAATATAAATAGTAGTGATAAAAAATAAAATGTTGAAACGACC
.....
TTAATTTTTATTGGTAGCAATATAAATAGTAGTGATAAAAAATAAAATGTTGAAACGACC
2230 2240 2250 2260 2270 2280

2290 2300 2310 2320 2330 2340
806559 TTATTCCAACATGCCATTACTCCAACATTAAATACCCTTTGGATTAATGGACAAAAGATA
.....
TTATTCCAACATGCCATTACTCCAACATTAAATACCCTTTGGATTAATGGACAAAAGATA
2290 2300 2310 2320 2330 2340

2350 2360 2370 2380 2390 2400
806559 GAAAACATGCCTTATCAAACAACACTTCAACAAGGTGATTGGTTAATTGATAGCAATGGC
.....
GAAAACATGCCTTATCAAACAACACTTCAACAAGGTGATTGGTTAATTGATAGCAATGGC
2350 2360 2370 2380 2390 2400

2410 2420 2430 2440 2450 2460
806559 AATGGTTACTTAAATTACTCAAGCAGAAAAAGTAAATGTAAGTCGCCAACATCAGGTTTCA
.....
AATGGTTACTTAAATTACTCAAGCAGAAAAAGTAAATGTAAGTCGCCAACATCAGGTTTCA
2410 2420 2430 2440 2450 2460

2470 2480 2490 2500 2510 2520
806559 GCGGAAAATAAAAATCGCCAACCGACAGAAGGAACTTTAGCTCGGCATGGATCGATCAC
.....
GCGGAAAATAAAAATCGCCAACCGACAGAAGGAACTTTAGCTCGGCATGGATCGATCAC
2470 2480 2490 2500 2510 2520

2530 2540 2550 2560 2570 2580
806559 AGCACTCGCCCCAAAGATGCCAGTTATGAGTATATGGTCTTTTTAGATGCGACACCTGAA
.....

The sequence identity at the amino acid level is shown below:

SEQ ID NO: 29 Present Invention Chondroitinase ABC I protein

44

10 20 30 40 50 60
365019 ATSNPAFDPKNLMQSEIYHFAQNNPLADFSSDKNSILTLSDKRSIMGNQSLWKKWKGSS

- ATSNPAFDPKNLMQSEIYHFAQNNPLADFSSDKNSILTLSDKRSIMGNQSLWKKWKGSS
10 20 30 40 50 60

70 80 90 100 110 120
365019 FTLHKKLIVPTDKEASKAWGRSSTPVFSFWLYNEKPIDGYLTIDFGEKLISTSEAQAGFK

- FTLHKKLIVPTDKEASKAWGRSSTPVFSFWLYNEKPIDGYLTIDFGEKLISTSEAQAGFK
70 80 90 100 110 120

130 140 150 160 170 180
365019 VKLDFTGWRTVGVSLNNDLENREMTLNATNTSSDGTQDSIGRSLGAKVDSIRFKAPSNVS

- VKLDFTGWRAVGVS LNNDLENREMTLNATNTSSDGTQDSIGRSLGAKVDSIRFKAPSNVS
130 140 150 160 170 180

190 200 210 220 230 240
365019 QGEIYIDRIMFSVDDARYQWSDYQVKTRLSEPEIQFHNVPQLPVT PENLAAIDLIRQRL

- QGEIYIDRIMFSVDDARYQWSDYQVKTRLSEPEIQFHNVPQLPVT PENLAAIDLIRQRL
190 200 210 220 230 240

250 260 270 280 290 300
365019 INEFGGEEKETNLALEENISKLKSDFDALNHTLANGGTQGRHLITDKQIIYQPENLNS

- INEFGGEEKETNLALEENISKLKSDFDALNIHTLANGGTQGRHLITDKQIIYQPENLNS
250 260 270 280 290 300

310 320 330 340 350 360
365019 QDKQLFDNYVILGNYTTLMFNISRAYVLEKDPTQKAQLKQMYLLMTKHLLDQGFVKGSAL

- QDKQLFDNYVILGNYTTLMFNISRAYVLEKDPTQKAQLKQMYLLMTKHLLDQGFVKGSAL
310 320 330 340 350 360

370 380 390 400 410 420
365019 VTTHHWGYSSRWYISTLLMSDALKEANLQTQVYDSLLWYSREFKSSFDMKVSADSSDLD

- VTTHHWGYSSRWYISTLLMSDALKEANLQTQVYDSLLWYSREFKSSFDMKVSADSSDLD

370 380 390 400 410 420

430 440 450 460 470 480

365019 YFNTLSRQHLALLLLEPDDQKRINLVNTFSHYITGALTQVPPGGKDGLRPDGTAWRHEGN
.....
_ YFNTLSRQHLALLLLEPDDQKRINLVNTFSHYITGALTQVPPGGKDGLRPDGTAWRHEGN
430 440 450 460 470 480

490 500 510 520 530 540

365019 YPGYSFPAFKNASQLIYLLRDTPFVSGESGWNSLKKAMVSAWIYSNPEVGLPLAGRHPN
.....
_ YPGYSFPAFKNASQLIYLLRDTPFVSGESGWNNLKKAMVSAWIYSNPEVGLPLAGRHPFN
490 500 510 520 530 540

550 560 570 580 590 600

365019 SPSLKSAQGYWLAWSAKSSPDKTLASIYLAISDKTQNESTAIFGETITPASLPQGFYA
.....
_ SPSLKSAQGYWLAWSAKSSPDKTLASIYLAISDKTQNESTAIFGETITPASLPQGFYA
550 560 570 580 590 600

610 620 630 640 650 660

365019 FNGGAFGIHRWQDKMVTCLKAYNTNVWSSEIYNKDNRYGRYQSHGVAQIVSNGSQLSQGYQ
.....
_ FNGGAFGIHRWQDKMVTCLKAYNTNVWSSEIYNKDNRYGRYQSHGVAQIVSNGSQLSQGYQ
610 620 630 640 650 660

670 680 690 700 710 720

365019 QEGWDWNRMPGATTIHLPLKDLDSPKPHTLMQRGERGFSGTSSLEGQYGMMAFDLIYPAN
.....
_ QEGWDWNRMPGATTIHLPLKDLDSPKPHTLMQRGERGFSGTSSLEGQYGMMAFDLIYPAN
670 680 690 700 710 720

730 740 750 760 770 780

365019 LERFDPNFTAKKSVLAADNHLIFIGSNINSSDKNKNVETTLFQHAIPTLNTLWINGQKI
.....
_ LERFDPNFTAKKSVLAADNHLIFIGSNINSSDKNKNVETTLFQHAIPTLNTLWINGQKI
730 740 750 760 770 780

790 800 810 820 830 840

365019 ENMPYQTTLQQGDWLIDSNNGNYLITQAEKVNVSQRHQVSAENKNRQPTEGNFSSAWIDH

.....
- ENMPYQTTLQQGDWLIDSNNGNGYLITQAEKVNVSQRHQVSAENKNRQPTTEGNFSSAWIDH
790 800 810 820 830 840

850 860 870 880 890 900
365019 STRPKDASYEYMVFLDATPEKMGEMAQKFRENNGLYQVLRKDKDVHIILDKLSNVTGYAF
.....
- STRPKDASYEYMVFLDATPEKMGEMAQKFRENNGLYQVLRKDKDVHIILDKLSNVTGYAF
850 860 870 880 890 900

910 920 930 940 950 960
365019 YQPASIEDKWIKKVNKPAIVMTHRQKDTLIVSAVTPDLNMTRQKAATPVTINVTINGKWQ
.....
- YQPASIEDKWIKKVNKPAIVMTHRQKDTLIVSAVTPDLNMTRQKAATPVTINVTINGKWQ
910 920 930 940 950 960

970 980 990
365019 SADKNSEVKYQVSGDNTELTFSTSYFGIPQEIKLSPLP
.....
- SADKNSEVKYQVSGDNTELTFSTSYFGIPQEIKLSPLP
970 980 990

REFERENCES

1. Fethiere J, Eggimann B, Cygler M (1999) Crystal structure of chondroitin AC lyase, a representative of a family of glycosaminoglycan degrading enzymes. J Mol Biol. 288:635-47.
2. Pojasek K, Shriver Z, Kiley, P Venkataraman G and Sasisekharan R. (2001) Biochem Biophys Res Commun. 286:343-51.
3. Huang W, Matte A, Li Y, Kim YS, Linhardt RJ, Su H, Cygler M. (1999) Crystal structure of chondroitinase B from Flavobacterium heparinum and its complex with a disaccharide product at 1.7 Å resolution. J Mol Biol. 294:1257-69.
4. Miura RO, Yamagata S, Miura Y, Harada T and Yamagata T. (1995) Anal Biochem. 225:333-40.
5. Yamagata T, Saito H, Habuchi O and Suzuki S. (1968) J Biol Chem. 243:1536-42.

[0089] Although the present invention has been described in considerable detail with reference to certain preferred embodiments thereof, other versions are possible. Therefore the spirit and scope of the appended claims should not be limited to the description and the preferred versions contain within this specification.

CLAIMS:

What is claimed is:

1. An composition comprising:

an isolated nucleic acid comprising a sequence that encodes for mutant proteoglycan degrading polypeptide.
2. The composition of claim 1 wherein the nucleic acid encodes for a proteoglycan degrading polypeptide that is a mutant of chondroitinase ABC Type I, (**SEQ ID NO: 1**), Chondroitinase ABC Type II, (**SEQ ID NO: 27**), Chondroitinase AC, (**SEQ ID NO: 5**), and Chondroitinase B, (**SEQ ID NO: 12**), hyaluronidase 1, (**SEQ ID NO: 30**), hyaluronidase 2, (**SEQ ID NO: 31**), hyaluronidase 3, (**SEQ ID NO: 32**), or hyaluronidase 4, (**SEQ ID NO: 33**).
3. The composition of claim 1 wherein the nucleic acid encodes for a proteoglycan degrading polypeptide that is a mutant of chondroitinase ABC Type I, (**SEQ ID NO: 1**), Chondroitinase ABC Type II, (**SEQ ID NO: 27**), Chondroitinase AC, (**SEQ ID NO: 5**), and Chondroitinase B, (**SEQ ID NO: 12**)
4. The composition of claim 1 wherein the nucleic acid encodes for a proteoglycan degrading polypeptide that is a mutant of chondroitinase ABC Type I, (**SEQ ID NO: 1**).
5. The composition of claim 1 wherein the nucleic acid encodes for a proteoglycan degrading polypeptide that is a mutant of Chondroitinase ABC Type II, (**SEQ ID NO: 27**).
6. The composition of claim 1 wherein the sequence of said nucleic acid is at least 80%, identical to a nucleic acid sequence encoding for a mutant proteoglycan degrading polypeptide.
7. The composition of claim 1 wherein the nucleic acid encodes for a polypeptide that degrades a proteoglycan in a tissue of the central nervous system.
8. The composition of claim 1 wherein the nucleic acid encodes for a polypeptide that degrades a chondroitin sulfate proteoglycan.

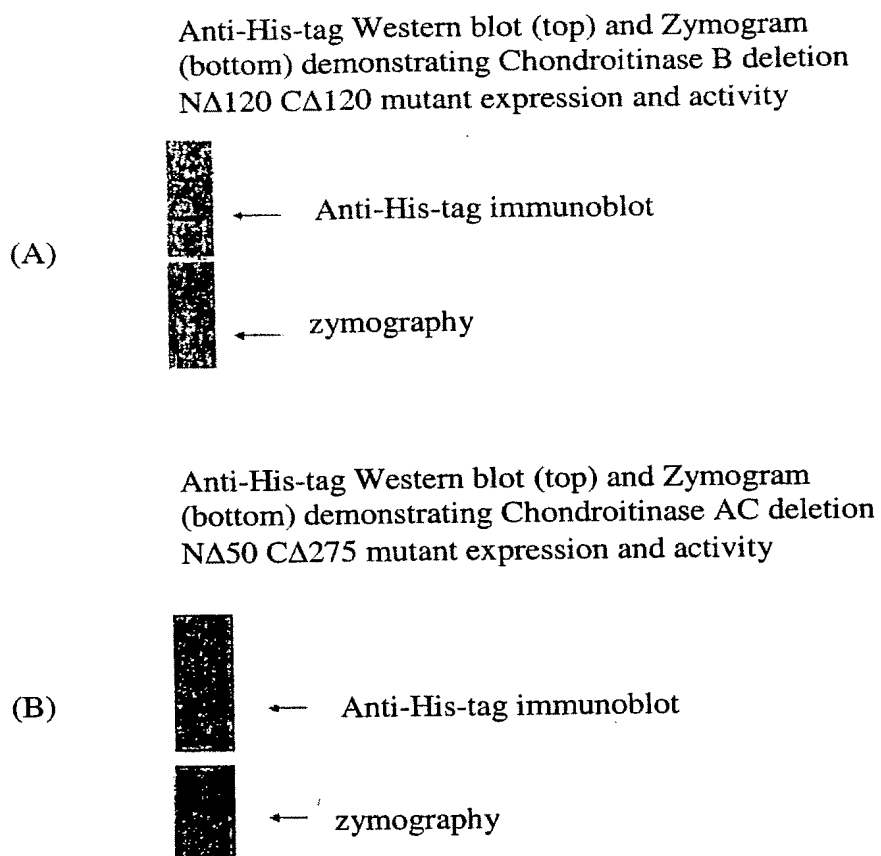
9. An expression vector comprising the nucleic acid of claim 1 operably linked to an expression control sequence.
10. The composition of claim 1 further including cells.
11. An composition comprising:

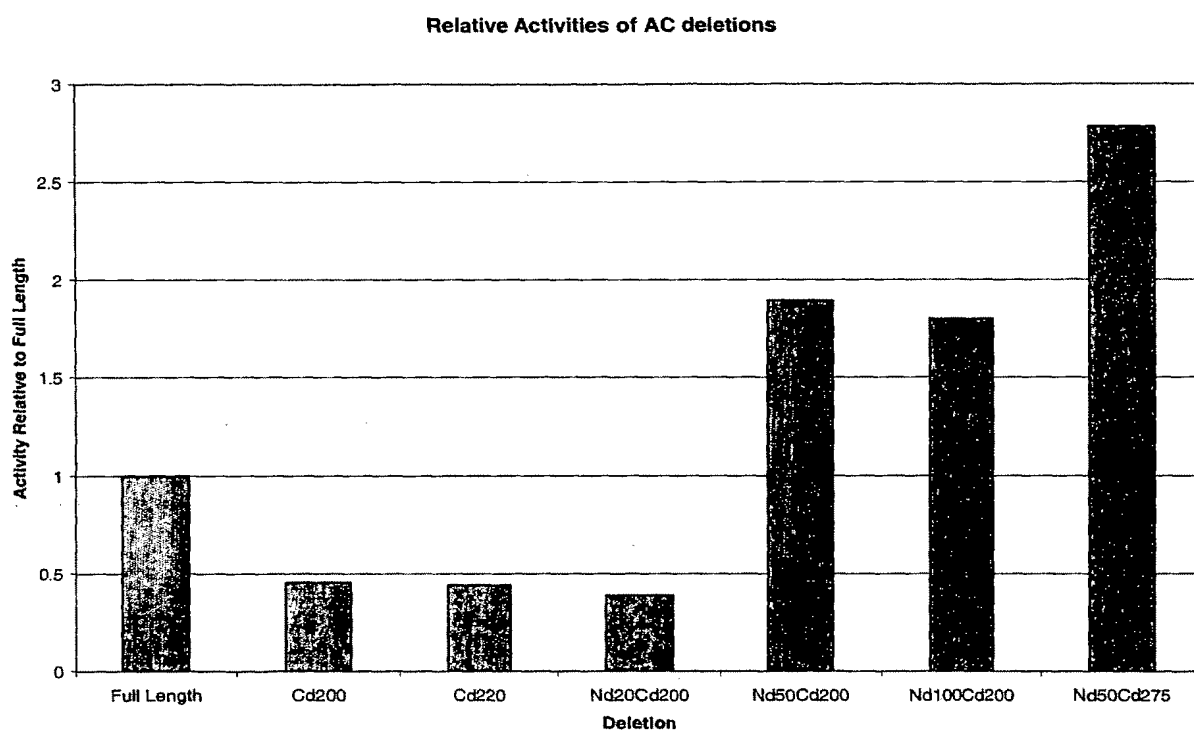
an isolated nucleic acid consisting of a sequence a sequence that encodes for a biologically active mutant proteoglycan degrading polypeptide.
12. The composition of claim 11 wherein the nucleic acid encodes for a proteoglycan degrading polypeptide that is a mutant of chondroitinase ABC Type I, (**SEQ ID NO: 1**), Chondroitinase ABC Type II, (**SEQ ID NO: 27**), Chondroitinase AC, (**SEQ ID NO: 5**), and Chondroitinase B, (**SEQ ID NO: 12**), hyaluronidase 1, (**SEQ ID NO: 30**), hyaluronidase 2, (**SEQ ID NO: 31**), hyaluronidase 3, (**SEQ ID NO: 32**), or hyaluronidase 4, (**SEQ ID NO: 33**).
13. The composition of claim 11 wherein the nucleic acid encodes for a proteoglycan degrading polypeptide that is a mutant of chondroitinase ABC Type I, (**SEQ ID NO: 1**), Chondroitinase ABC Type II, (**SEQ ID NO: 27**), Chondroitinase AC, (**SEQ ID NO: 5**), and Chondroitinase B, (**SEQ ID NO: 12**)
14. The composition of claim 11 wherein the nucleic acid encodes for a proteoglycan degrading polypeptide that is a mutant of chondroitinase ABC Type I, (**SEQ ID NO: 1**)
15. The composition of claim 11 wherein the nucleic acid encodes for a proteoglycan degrading polypeptide that is a mutant of Chondroitinase ABC Type II, (**SEQ ID NO: 27**).
16. The composition of claim 11 wherein the nucleic acid encodes for a polypeptide that degrades a proteoclycan in a tissue of the central nervous system.
17. The composition of claim 11 wherein the nucleic acid encodes polypeptide that degrades chondroitin sulfate proteoglycan.
18. The composition of claim 11 further including cells.

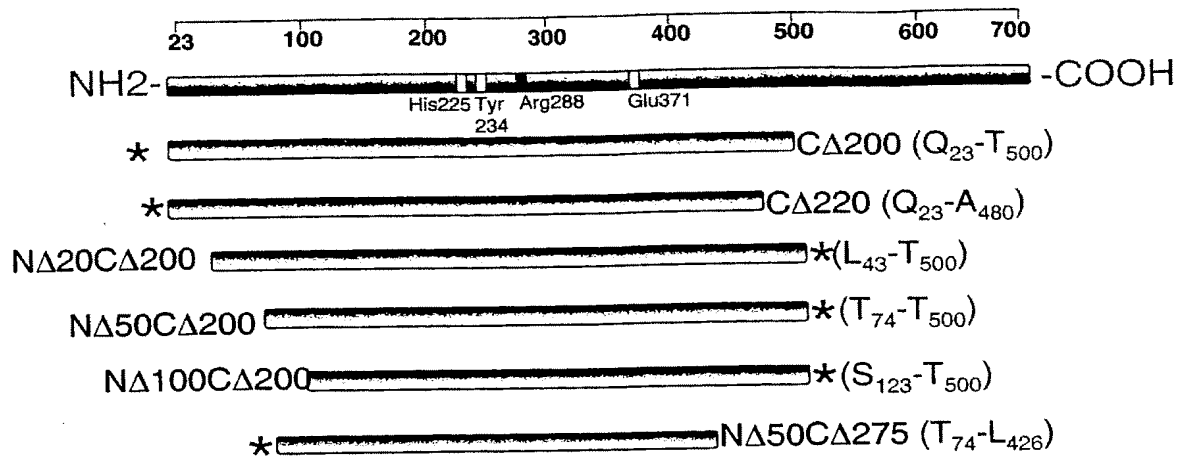
19. A composition comprising:
- a polypeptide comprising the amino acid sequence of a biologically active mutant proteoglycan degrading polypeptide.
20. The composition of claim 19 wherein the proteoglycan degrading polypeptide is a mutant of chondroitinase ABC Type I, (**SEQ ID NO: 1**), Chondroitinase ABC Type II, (**SEQ ID NO: 27**), Chondroitinase AC, (**SEQ ID NO: 5**), and Chondroitinase B, (**SEQ ID NO: 12**), hyaluronidase 1, (**SEQ ID NO: 30**), hyaluronidase 2, (**SEQ ID NO: 31**), hyaluronidase 3, (**SEQ ID NO: 32**), or hyaluronidase 4, (**SEQ ID NO: 33**).
21. The composition of claim 19 wherein the proteoglycan degrading polypeptide is a mutant of chondroitinase ABC Type I, (**SEQ ID NO: 1**), Chondroitinase ABC Type II, (**SEQ ID NO: 27**), Chondroitinase AC, (**SEQ ID NO: 5**), or Chondroitinase B, **SEQ ID NO: 12**).
22. The composition of claim 19 wherein the proteoglycan degrading polypeptide is a mutant of chondroitinase ABC Type I, (**SEQ ID NO: 1**).
23. The composition of claim 19 wherein the proteoglycan degrading polypeptide is a mutant of chondroitinase ABC Type I, (**SEQ ID NO: 27**).
24. The composition of claim 19 proteoglycan degrading polypeptide is (**SEQ ID NO: 2**), (**SEQ ID NO: 3**), or (**SEQ ID NO: 4**).
25. The composition of claim 19 wherein the polypeptide degrades a proteoglycan in a tissue of the central nervous system.
26. The composition of claim 19 wherein the polypeptide degrades a chondroitin sulfate proteoglycan.
27. The composition of claim 19 further including cells.
28. The composition of claim 19 and a pharmaceutically acceptable excipient.
29. The composition of claim 19 further including molecules which block the action of neurite growth inhibitors, molecules which promote neurite adhesion, diagnostic molecules or a combination of these.

30. A method of treating a tissue, the method comprising:
- administering a mutant proteoglycan degrading polypeptide composition to the tissue, said tissue including proteoglycan molecules, said composition degrading at least a portion of the proteoglycan in the tissue.
32. The method of claim 30 wherein the tissue is from the CNS.
33. The method of claim 30 wherein the proteoglycan degradation promotes diffusion of molecules into the tissue.
34. The method of claim 30 further comprising the act of identifying tissue from a contusive spinal cord injury.
35. The method of claim 30 wherein the composition promote neurite regeneration.
36. The method of claim 30 wherein the composition further includes molecules which block the action of neurite growth inhibitors, molecules which promote neurite adhesion, diagnostic molecules or a combination of these.
37. The method of claim 30 wherein the plasticity of the of the nervous system is improved.
38. The method of claim 30 wherein the proteoglycan degrading polypeptide is a mutant of chondroitinase ABC Type I, (**SEQ ID NO: 1**), Chondroitinase ABC Type II, (**SEQ ID NO: 27**), Chondroitinase AC, (**SEQ ID NO: 5**), and Chondroitinase B, (**SEQ ID NO: 12**), hyaluronidase 1, (**SEQ ID NO: 30**), hyaluronidase 2, (**SEQ ID NO: 31**), hyaluronidase 3, (**SEQ ID NO: 32**), or hyaluronidase 4, (**SEQ ID NO: 33**).
39. The method of claim 30 wherein the proteoglycan degrading polypeptide is a mutant of chondroitinase ABC Type I, (**SEQ ID NO: 1**), Chondroitinase ABC Type II, (**SEQ ID NO: 27**), Chondroitinase AC, (**SEQ ID NO: 5**), or Chondroitinase B, **SEQ ID NO: 12**).
40. The method of claim 30 wherein the proteoglycan degrading polypeptide is a mutant of chondroitinase ABC Type I, (**SEQ ID NO: 1**).

41. The method of claim 30 wherein the proteoglycan degrading polypeptide is a mutant of chondroitinase ABC Type I, (SEQ ID NO: 27).
42. The method of claim 30 proteoglycan degrading polypeptide is (SEQ ID NO: 2), (SEQ ID NO: 3), or (SEQ ID NO: 4).
43. A purified chondroitinase mutant polypeptide that degrades a proteoglycan.

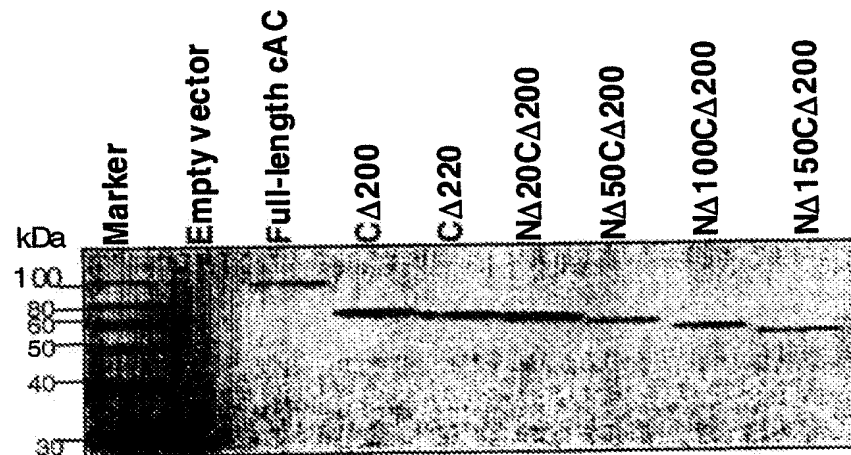
**FIG. 1**

**FIG. 2**



(A)

Confirmation of Expression of Chondroitinase AC Deletion Mutants by Western Blotting



(B)

FIG. 3

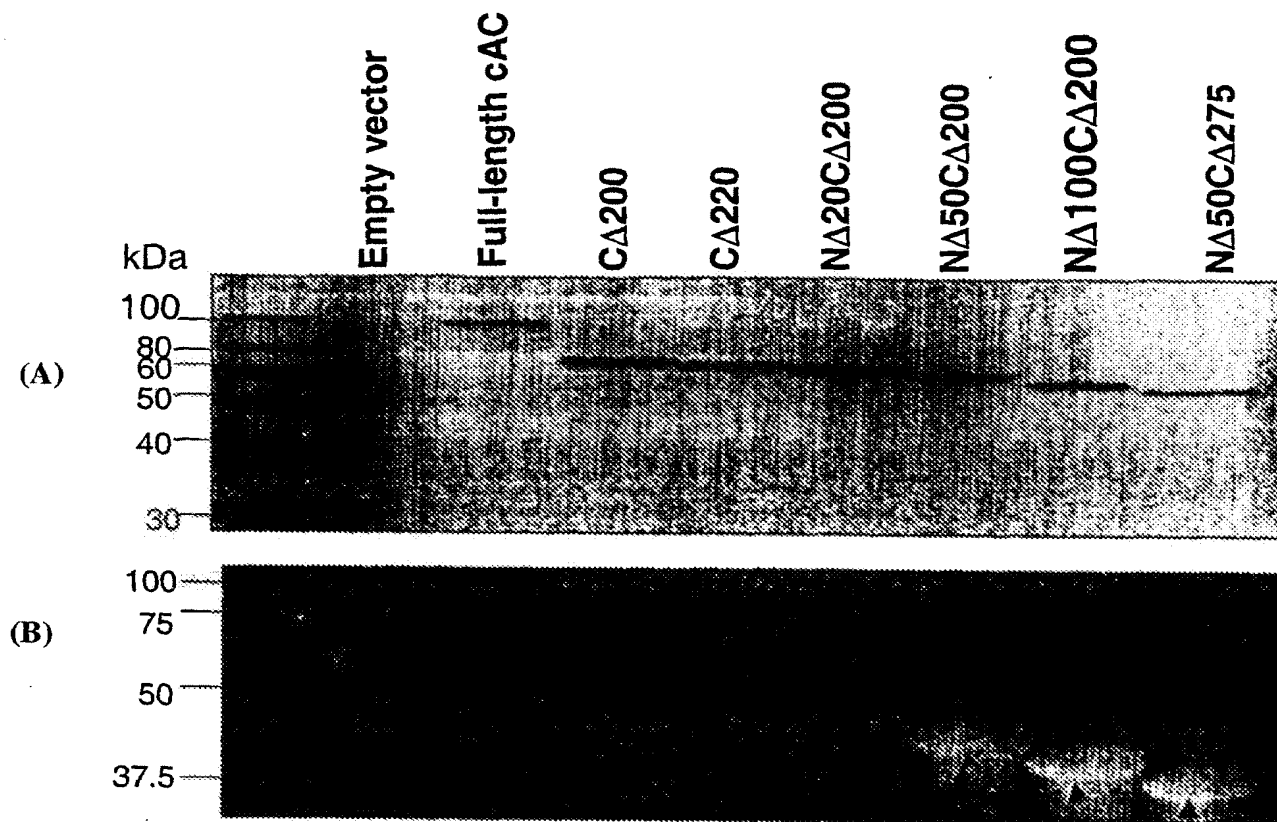


FIG. 4

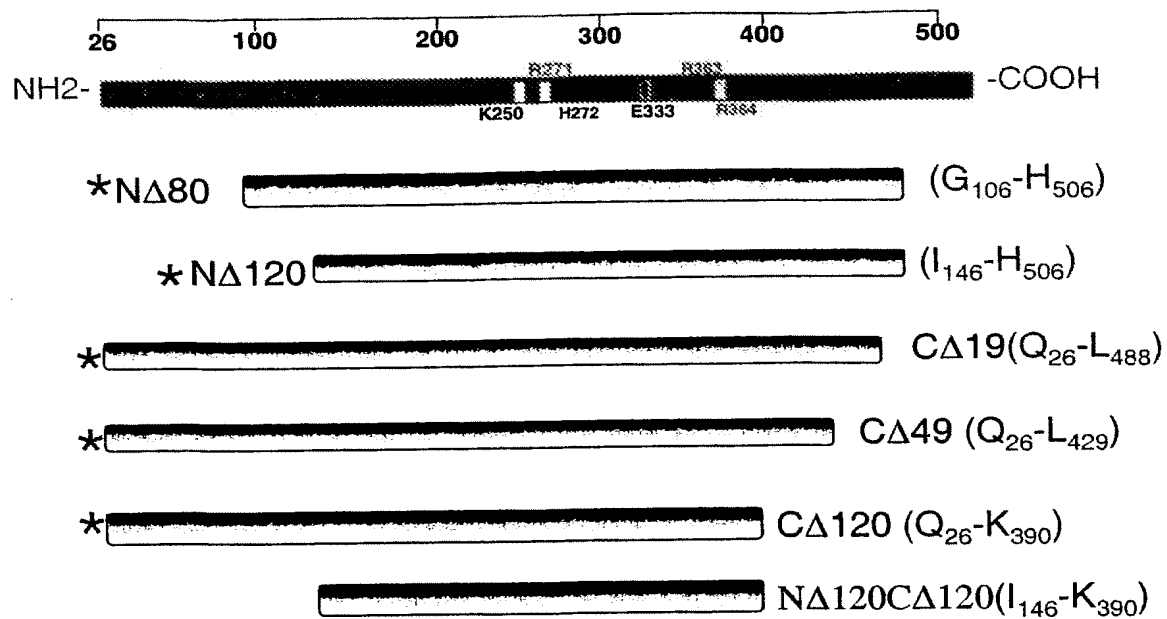


FIG. 5

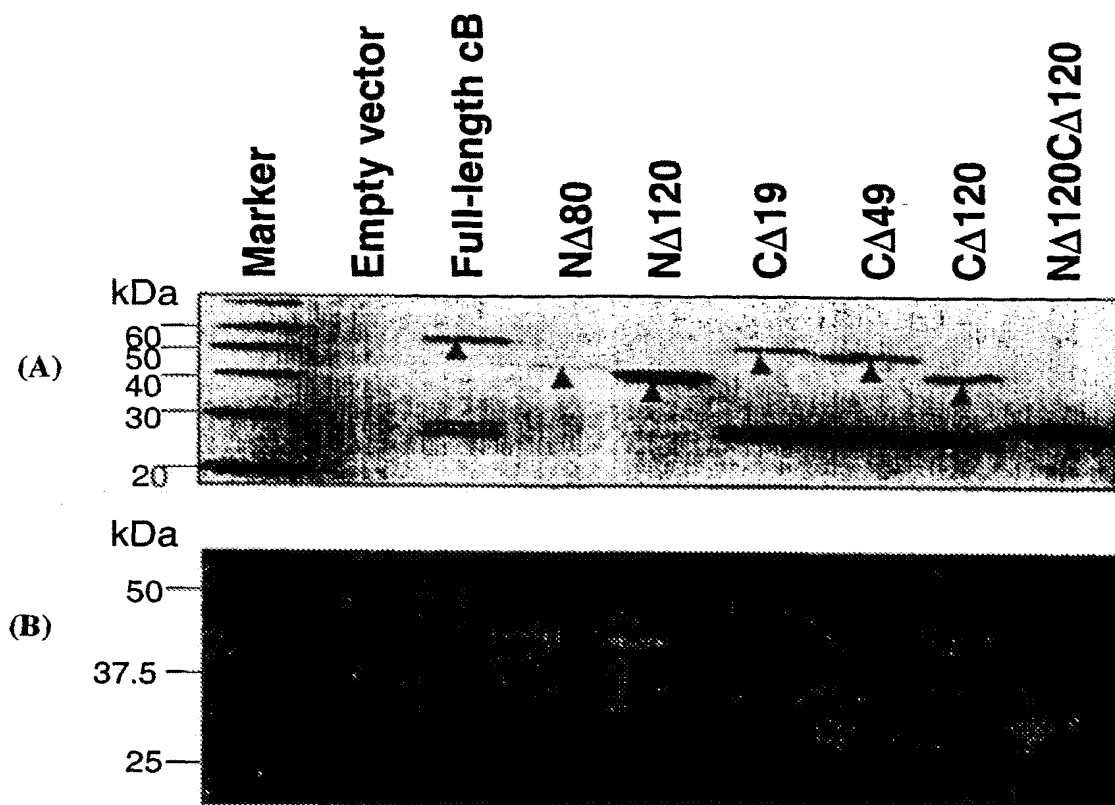


FIG. 6

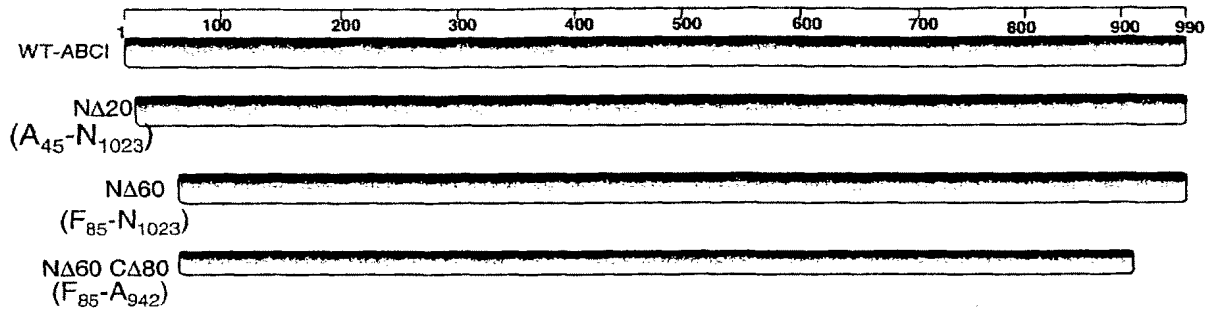


FIG. 7

SEQUENCE LISTING

SEQ ID NO: 1 Chondroitinase ABC I protein

ORIGIN

atsnpa fdpknlmqse iyhfaqqnpl adfssdknsi
 61 ltlsdkrsim gnqslwkwwk ggssftlhkk livptdkeas kawgrsstpv fsfwlynekp
 121 idgyltidfg eklistseaq agfkvkldft gwrTvgvsln ndlenremtl natntssdgt
 181 qdsigrslga kvdsirfkp snvsqgeiyi drimfsvdda ryqwsdyqvk trlsepeiqf
 241 hnvkpqlpvt penlaaidli rqrlinefvg geketnlale enisklksdf dalnThtlan
 301 ggtqgrhlit dkqiiyqpe nlmsqdkqlf dnyvilgnyt tlmfnisray vlekdpqka
 361 qlkqmyllmt khldqgfvk gsalvtthhw gyssrwwyis tlmsdalke anlqtqvys
 421 llwysrefks sfdmkvsads sdldyfntls rqlalalle pddqkrinlv ntfshtyga
 481 ltqvppggkd glrpdgtawr hegnypgysf pafknaqli yllrdtpfsv gesgwlnlkk
 541 amvsawiysn pevglplagr hpfnsplks vaqgyywlam saksspdktl asiylaisdk
 601 tqnestaifg etitpaslpq gfyafnggaf gihrwqdkmv tlkayntnvw sseyinkdnr
 661 ygryqshgva qivsngsqs qgyqqegwdw nrmegattih lplkdldspk phtlmqrger
 721 gfsqtssleg qygmmafni ypanlerfdp nftakksvla adnhlifigs ninssdknkn
 781 vetltfghai tptlntlw in gqkienmpyq tllqqgdwli dsngngylit qaekvnvsrq
 841 hqvsakenr qptegnfsa widhstrpkd asyeymvfld atpekmngema qkfrenngly
 901 qvlrkdvdh iildklsnvt gyafyqpasi edkwikkvnk paivmthrqk dtlvsavtp
 961 dlnmtrqkaa tpvtinvtn gkwqsadkns evkyqvsgdn teltfisyfg ipqeiklspl
 1021 p

SEQ ID NO: 2 NA20 ABCI (A₄₅-N₁₀₂₃), protein

aqnnpl adfssdknsi
 61 ltlsdkrsim gnqslwkwwk ggssftlhkk livptdkeas kawgrsstpv fsfwlynekp
 121 idgyltidfg eklistseaq agfkvkldft gwrTvgvsln ndlenremtl natntssdgt
 181 qdsigrslga kvdsirfkp snvsqgeiyi drimfsvdda ryqwsdyqvk trlsepeiqf
 241 hnvkpqlpvt penlaaidli rqrlinefvg geketnlale enisklksdf dalnhtlan
 301 ggtqgrhlit dkqiiyqpe nlmsqdkqlf dnyvilgnyt tlmfnisray vlekdpqka
 361 qlkqmyllmt khldqgfvk gsalvtthhw gyssrwwyis tlmsdalke anlqtqvys
 421 llwysrefks sfdmkvsads sdldyfntls rqlalalle pddqkrinlv ntfshtyga
 481 ltqvppggkd glrpdgtawr hegnypgysf pafknaqli yllrdtpfsv gesgwlnlkk
 541 amvsawiysn pevglplagr hpfnsplks vaqgyywlam saksspdktl asiylaisdk
 601 tqnestaifg etitpaslpq gfyafnggaf gihrwqdkmv tlkayntnvw sseyinkdnr
 661 ygryqshgva qivsngsqs qgyqqegwdw nrmegattih lplkdldspk phtlmqrger
 721 gfsqtssleg qygmmafni ypanlerfdp nftakksvla adnhlifigs ninssdknkn
 781 vetltfghai tptlntlw in gqkienmpyq tllqqgdwli dsngngylit qaekvnvsrq
 841 hqvsakenr qptegnfsa widhstrpkd asyeymvfld atpekmngema qkfrenngly
 901 qvlrkdvdh iildklsnvt gyafyqpasi edkwikkvnk paivmthrqk dtlvsavtp
 961 dlnmtrqkaa tpvtinvtn gkwqsadkns evkyqvsgdn teltfisyfg ipqeiklspl
 1021 p

SEQ ID NO: 3 NΔ60 ABCI (F₈₅-N₁₀₂₃) protein

flhkklivptdkeas kawgrsstpv fsfwlynekp
 121 idgyltidfg eklistseaq agfkvkl dft gwrvtgvsl nndlenremtl natntssdgt
 181 qdsigrslga kvdsirfkp snvsqgeiyi drimfsvdda ryqwsdyqvk trlsepeiqf
 241 hnvkpqlpvt penlaaidli rqrlinefvg geketnlale enisklksdf dalnthtlan
 301 ggtqgrhlit dkqiiyqpe nlnsqdkqlf dnyvilgnyt tlmfnisray vlekdpqka
 361 qlkqmyllmt khlldqgf vk gsalvtthhw gyssrwwyis tlmsdalke anlqtqvys
 421 llwysrefks sfdmkvsads sldyfntls rqlalalle pddqkrinlv ntfshtyitga
 481 ltqvppggkd glrpdgtawr hegnypgysf pafknsqli yllrdtpfsv gesgwnnlk
 541 amvsawiysn pevglplagr hpfnsplks vaqgyywlam saksspdktl asiylaisdk
 601 tqnestaifg etitpaslpq gfyafnggaf gihrwqdkmv tlkayntnvw sseiyndnr
 661 ygryqshgva qivsngsqs qgyqqegwdw nrmegattih lplkdldspk phtlmqrger
 721 gfsgtssleg qygmmafnli ypanlerfdp nftakksvla adnhlifigs ninssdknkn
 781 vetltfghai tptlntlwin gqkienmpyq tlqqgdwli dsngngylit qaekvnvsrq
 841 hqvsakenr qptegnfsa widhstrpkd asyeymvfld atpekmgema qkfrenngly
 901 qvlrkdvdh iildklsnvt gyafyqpasi edkwikkvkn paivmthrqk dtlivsavtp
 961 dlnmtrkaa tptinvtn gkwqsadkns evkyqvsgdn teltfisyf ipqeiklspl
 1021 p

SEQ ID No. 4: NΔ60 CΔ80 ABCI (F₈₅-A₉₄₂) protein

flhkklivptdkeas kawgrsstpv fsfwlynekp
 121 idgyltidfg eklistseaq agfkvkl dft gwrvtgvsl nndlenremtl natntssdgt
 181 qdsigrslga kvdsirfkp snvsqgeiyi drimfsvdda ryqwsdyqvk trlsepeiqf
 241 hnvkpqlpvt penlaaidli rqrlinefvg geketnlale enisklksdf dalnthtlan
 301 ggtqgrhlit dkqiiyqpe nlnsqdkqlf dnyvilgnyt tlmfnisray vlekdpqka
 361 qlkqmyllmt khlldqgf vk gsalvtthhw gyssrwwyis tlmsdalke anlqtqvys
 421 llwysrefks sfdmkvsads sldyfntls rqlalalle pddqkrinlv ntfshtyitga
 481 ltqvppggkd glrpdgtawr hegnypgysf pafknsqli yllrdtpfsv gesgwnnlk
 541 amvsawiysn pevglplagr hpfnsplks vaqgyywlam saksspdktl asiylaisdk
 601 tqnestaifg etitpaslpq gfyafnggaf gihrwqdkmv tlkayntnvw sseiyndnr
 661 ygryqshgva qivsngsqs qgyqqegwdw nrmegattih lplkdldspk phtlmqrger
 721 gfsgtssleg qygmmafnli ypanlerfdp nftakksvla adnhlifigs ninssdknkn
 781 vetltfghai tptlntlwin gqkienmpyq tlqqgdwli dsngngylit qaekvnvsrq
 841 hqvsakenr qptegnfsa widhstrpkd asyeymvfld atpekmgema qkfrenngly
 901 qvlrkdvdh iildklsnvt gyafyqpasi edkwikkvkn pa

SEQ ID NO: 5 Chondroitinase AC protein Locus 1HMW_A**ORIGIN**

1 mkklfvtciv ffsilspall iaqqtgtael imkrvmldlk kplrnmdkva eknltlqpd
 61 gswkdvpkyd damtnwlpnn hllqletii ayiekdshyy gddkvfdqis kafkywydsd
 121 pksrnwwhne iatpqaigem lilmrygkpk ldeavhkl ermkrgepek ktganktdia
 181 lhyfyralit sdeallsfav kelfypvqv hyeeglqydy sylqhgplq issygavfit
 241 gvlklanyvr dtpyalstek laifskyyrd sylkaigrsy mdfnvegrgv srpdilnkka
 301 ekkrlrvakm idlkhteewa daiartdstv aagykiepyh hqfwngdyvq hlrpaysfnv
 361 rmvskrttrs esgkenllg rylsdgatni qlrgpeyyi mpvwewdkip gitsrdyld
 421 rpltklwgeq gsndfaggvs dgvygasaya ldydqlakk awfffdkeiv clgaginsna
 481 penittlnq swlmgpvist agktgrgkit tfkaqqqfwl lhdaigyyfp eganlslstq

541 sqkgnwfhin nshskdevsg dvfklwinhg arpenaqyay ivlpginkpe eikkyngtap
 601 kvlantnqlq avyhhqldmv qaiifytagkl svagieietd kpcavlikhi ngkqviwaad
 661 plqkektavl sirdlktgkt nrvkidfpqk efagatvelk

SEQ ID NO: 6 CA200 AC (Q₂₃-T₅₀₀) protein

qqtgtael imkrvmldlk kplrnmdkva eknlnlqlpd
 61 gswkdvpkyd damtnwlpnn hllqletiiq ayiekdshyy gddkvfdqis kafkywydsd
 121 pksrnwwhne iatpqalgem lilmrygkpk idealvhklt ermkrgepek ktganktdia
 181 lhyfyrallt sdeallsfav kelfypvqfv hyeeglqydy sylqhgpqlq issygavfit
 241 gvlklanyvr dtpyalstek laifskyyrd sylkairgsy mdfnvegrgv srpdilnkka
 301 ekkrlvakm idlkhteewa daiartdstv aagykiepyh hqfwngdyvq hlrpaysfnv
 361 rmvsktrrs esgngenllg rylsdgatni qlrgpeyyini mpvwewdkip gitsrdyltd
 421 rpltklwgeq gsndfaggvs dgvygasaya ldydslqakk awfffdkeiv clgaginsna
 481 penittlnq swlmgpvist

SEQ ID NO: 7 CA220 AC (Q₂₃-A₄₈₀) protein

qqtgtael imkrvmldlk kplrnmdkva eknlnlqlpd
 61 gswkdvpkyd damtnwlpnn hllqletiiq ayiekdshyy gddkvfdqis kafkywydsd
 121 pksrnwwhne iatpqalgem lilmrygkpk idealvhklt ermkrgepek ktganktdia
 181 lhyfyrallt sdeallsfav kelfypvqfv hyeeglqydy sylqhgpqlq issygavfit
 241 gvlklanyvr dtpyalstek laifskyyrd sylkairgsy mdfnvegrgv srpdilnkka
 301 ekkrlvakm idlkhteewa daiartdstv aagykiepyh hqfwngdyvq hlrpaysfnv
 361 rmvsktrrs esgngenllg rylsdgatni qlrgpeyyini mpvwewdkip gitsrdyltd
 421 rpltklwgeq gsndfaggvs dgvygasaya ldydslqakk awfffdkeiv clgaginsna

SEQ ID NO: 8 NA20 CA200 AC (L₄₃-T₅₀₀) protein

lrmmdkva eknlnlqlpd
 61 gswkdvpkyd damtnwlpnn hllqletiiq ayiekdshyy gddkvfdqis kafkywydsd
 121 pksrnwwhne iatpqalgem lilmrygkpk idealvhklt ermkrgepek ktganktdia
 181 lhyfyrallt sdeallsfav kelfypvqfv hyeeglqydy sylqhgpqlq issygavfit
 241 gvlklanyvr dtpyalstek laifskyyrd sylkairgsy mdfnvegrgv srpdilnkka
 301 ekkrlvakm idlkhteewa daiartdstv aagykiepyh hqfwngdyvq hlrpaysfnv
 361 rmvsktrrs esgngenllg rylsdgatni qlrgpeyyini mpvwewdkip gitsrdyltd
 421 rpltklwgeq gsndfaggvs dgvygasaya ldydslqakk awfffdkeiv clgaginsna
 481 penittlnq swlmgpvist

SEQ ID NO: 9 NA50 CA200 AC (T₇₄-T₅₀₀) protein

tnwlpnn hllqletiiq ayiekdshyy gddkvfdqis kafkywydsd
 121 pksrnwwhne iatpqalgem lilmrygkpk idealvhklt ermkrgepek ktganktdia
 181 lhyfyrallt sdeallsfav kelfypvqfv hyeeglqydy sylqhgpqlq issygavfit
 241 gvlklanyvr dtpyalstek laifskyyrd sylkairgsy mdfnvegrgv srpdilnkka
 301 ekkrlvakm idlkhteewa daiartdstv aagykiepyh hqfwngdyvq hlrpaysfnv
 361 rmvsktrrs esgngenllg rylsdgatni qlrgpeyyini mpvwewdkip gitsrdyltd
 421 rpltklwgeq gsndfaggvs dgvygasaya ldydslqakk awfffdkeiv clgaginsna
 481 penittlnq swlmgpvist

SEQ ID NO: 10 NA100 CA200 AC (S₁₂₃-T₅₀₀) protein

srnwwhne iatpqalgem lilmrygkpk ldealvhklt ermkrgepek ktganktdia
 181 lhyfyrallt sdeallsfav kelfypvqfv hyeeglqydy sylqhgplq issygavfit
 241 gvlklanyvr dtpyalstek laifskyyrd sylkairgsy mdfnvegrgv srpdilnkka
 301 ekkrlrvakm idlkhteewa daiartdstv aagykiepyh hqfwngdyvq hlrpaysfnv
 361 rmvsktrtrs esgkenllg rylsdgatni qlrgpeyyini mpvwewdkip gitsrdyltd
 421 rpltklwgeq gsndfaggvs dgvygasaya ldydslqakk awfffdkeiv clgaginsna
 481 penittlnq swlmgpvist

SEQ ID NO: 11 NA50 CA275 AC (T₇₄-L₄₂₆) protein

tnwlpnn hllqletiiq ayiekdshyy gddkvfdqis kafkywydsd
 121 pksrnwwhne iatpqalgem lilmrygkpk ldealvhklt ermkrgepek ktganktdia
 181 lhyfyrallt sdeallsfav kelfypvqfv hyeeglqydy sylqhgplq issygavfit
 241 gvlklanyvr dtpyalstek laifskyyrd sylkairgsy mdfnvegrgv srpdilnkka
 301 ekkrlrvakm idlkhteewa daiartdstv aagykiepyh hqfwngdyvq hlrpaysfnv
 361 rmvsktrtrs esgkenllg rylsdgatni qlrgpeyyini mpvwewdkip gitsrdyltd
 421 rpltkl

SEQ ID NO: 12 Chondroitinase B Locus Q46079 protein**ORIGIN**

1 mkmlnlklagy lpimvllnv apclgqvvas netlyqvcke vkpgglvqia dgtykdvqli
 61 vsnsgksglp itikalnpgk vfftdakve lrgehlileg iwfkdgndrai qawkshgpgl
 121 vaiygsynri tacvdfcfde ansayittsl tedgkvpqhc ridhcsftdk itfdqvinln
 181 ntaraikdgs vggpgmyhrv dhcfsnpqk pgnagggi gyyrndigrc lvdsnlfmrq
 241 dseaeiitsk sqenvyygnt ylnsqgtmnf rhgdhqvain nfyigndqrf gyggmfvwgs
 301 rhviacnyfe lsetiksrn aalylnpgam asehalafdm liannafinv ngaihfnp
 361 derrkeycaa nrlkfetphq lmlkgnlffk dkpyvypffk ddyfiagkns wtgnvalgve
 421 kgipvnisan rsaykpvkik diqpiegial dlnaliskgi tgpplswdev rpywlkempg
 481 tyaltarlsa draakfkavi krnkeh

SEQ ID NO: 13 NA80 Chase B (G₁₀₆-H₅₀₆) protein

gnrai qawkshgpgl
 121 vaiygsynri tacvdfcfde ansayittsl tedgkvpqhc ridhcsftdk itfdqvinln
 181 ntaraikdgs vggpgmyhrv dhcfsnpqk pgnagggi gyyrndigrc lvdsnlfmrq
 241 dseaeiitsk sqenvyygnt ylnsqgtmnf rhgdhqvain nfyigndqrf gyggmfvwgs
 301 rhviacnyfe lsetiksrn aalylnpgam asehalafdm liannafinv ngaihfnp
 361 derrkeycaa nrlkfetphq lmlkgnlffk dkpyvypffk ddyfiagkns wtgnvalgve
 421 kgipvnisan rsaykpvkik diqpiegial dlnaliskgi tgpplswdev rpywlkempg
 481 tyaltarlsa draakfkavi krnkeh

SEQ ID NO: 14 NA120 Chase B (I₁₄₆-H₅₀₆) protein

ittsl tedgkvpqhc ridhcsftdk itfdqvinln
 181 ntaraikdgs vggpgmyhrv dhcfsnpqk pgnagggi gyyrndigrc lvdsnlfmrq
 241 dseaeiitsk sqenvyygnt ylnsqgtmnf rhgdhqvain nfyigndqrf gyggmfvwgs
 301 rhviacnyfe lsetiksrn aalylnpgam asehalafdm liannafinv ngaihfnp

361 derrickycaa nrlkfetphq lmlkgnlffk dkpyvypffk ddyfiagkns wtgnvalgve
 421 kgipvnisan rsaykpvkik diqpiegial dlnaliskgi tgkplswdev rpywlkempg
 481 tyaltarl

SEQ ID NO: 15 CA19 Chase B (Q₂₆-L₄₈₈) protein

qvvas netlyqvcke vkpgglvqia dgtykdvqli
 61 vsnsgks glp itikalnpgk vfftdakve lrgehlileg iwfkdnrai qawkshgpgl
 121 vaiygsynri tacvfdfcde ansayittsl tedgkvpqhc ridhesftdk itfdqvinln
 181 ntaraidgs vggpgmyhrv dhcffsnpqk pgnagggi ggyrndigrc lvdsnlfnrq
 241 dseaeitsk sqenvvygnt ylncqgtmnf rhgdhqvain nfyigndqrf gyggmfvwgs
 301 rhviacnyfe lsetiksrn aalylnpgam asehalafdm liannafinv ngaihfnp
 361 derrickycaa nrlkfetphq lmlkgnlffk dkpyvypffk ddyfiagkns wtgnvalgve
 421 kgipvnisan rsaykpvkik diqpiegial dlnaliskgi tgkplswdev rpywlkempg
 481 tyaltarl

SEQ ID NO: 16 CA120 Chase B (Q₂₆-K₃₉₀) protein

qvvas netlyqvcke vkpgglvqia dgtykdvqli
 61 vsnsgks glp itikalnpgk vfftdakve lrgehlileg iwfkdnrai qawkshgpgl
 121 vaiygsynri tacvfdfcde ansayittsl tedgkvpqhc ridhesftdk itfdqvinln
 181 ntaraidgs vggpgmyhrv dhcffsnpqk pgnagggi ggyrndigrc lvdsnlfnrq
 241 dseaeitsk sqenvvygnt ylncqgtmnf rhgdhqvain nfyigndqrf gyggmfvwgs
 301 rhviacnyfe lsetiksrn aalylnpgam asehalafdm liannafinv ngaihfnp
 361 derrickycaa nrlkfetphq lmlkgnlffk

SEQ ID NO: 17 NA120 CA120 Chase B (I₁₄₆-K₃₉₀) protein

ittsl tedgkvpqhc ridhesftdk itfdqvinln
 181 ntaraidgs vggpgmyhrv dhcffsnpqk pgnagggi ggyrndigrc lvdsnlfnrq
 241 dseaeitsk sqenvvygnt ylncqgtmnf rhgdhqvain nfyigndqrf gyggmfvwgs
 301 rhviacnyfe lsetiksrn aalylnpgam asehalafdm liannafinv ngaihfnp
 361 derrickycaa nrlkfetphq lmlkgnlffk

SEQ ID NO: 18 Choindroitinase AC nucleotide locus CHU27583

ORIGIN

1 atgaagaaat tatttgaac ctgtatagtc ttttcteta ttttaagtcc tgctctgct
 61 attgcacagc agaccggtac tgcagaactg attatgaagc gggtgatgct ggacctaaa
 121 aagcctttgc gcaatatgga taagggtggcg gaaaagaacc tgaatacgt gcagcctgac
 181 ggtagctgga aggatgtgcc ttataaatgat gatgcatga ccaattggtt gccaaacaac
 241 cacctgctac aattggaaac tattatacag gcttatattg aaaaagatag tcactattat
 301 ggcgacgata aagtgttga ccagatttcc aaagcttta agtattggtg tgacagcgac
 361 ccgaaaagcc gcaactggtg gcacaaatga attgccactc cgcaggccct tggatgaatg
 421 ctgacctga tgcgttacgg taaaagccg ctgatgaag cattggtgca taaattgacc
 481 gaaagaatga agcggggcga accggagaag aaaacggggg ccaacaaaac agatcgcgc
 541 ctgcattact ttatcgtgc ttgttaacg tctgatgagg ctttgcttc ctgcgcgta
 601 aaagaattgt ttatcccg acagtttga cactatgagg aaggcctgca atacgattat
 661 tctacctgc agcaggtcc gcaattacag atacgagct acggtgccgt attattacc
 721 ggggtactga aacttgcaa ttacgttagg gataccctt atgctttaag taccgagaaa
 781 ctggctatat ttcaaagta ttaccgcgac agttatctga aagctatccg tggaagtat

841 atggatttta acgtagaagg ccgaggagta agccggccag acattctaaa taaaaaggca
 901 gaaaaaaga ggttgctggt ggcgaagatg atcgatctta agcactga agaattggct
 961 gatgcgatag ccaggacaga tagcacagtt gcggccggct ataagattga gccctatcac
 1021 catcagttct ggaatggtga ttatgtgcaa catttaagac ctgcctatc ttttaattgt
 1081 cgtatggtga gtaagcggac ccgacgcagt gaatccggca ataaagaaaa cctgctgggc
 1141 aggtatttat ctgatggggc tactaacata caattgcgcg gaccagaata ctataacatt
 1201 atgccgggtat gggaatggga caagattcct ggcataacca gccgtgatta ttaaccgac
 1261 agacctttga cgaagctttg gggagagcag gggagcaatg actttgcagg aggggtgtct
 1321 gatggtgtat acggggccag tgcctacgca ttggattacg atagcttaca ggcaaagaaa
 1381 gcctggttct ttttgacaa agagattgta tgccttggtg ccggtatcaa cagcaatgcc
 1441 cctgaaaaa ttaccactac ccttaaccag agctggttaa atggcccggt tataagtact
 1501 gcaggtaaaa ccggccgggg taaaataaca acgtttaag cacagggaca gttctggtg
 1561 ttgcacgatg cgattggtta ttactttcct gaaggggcca acctagtct gagtaccag
 1621 tcgcaaaaag gcaattggtt ccacatcaac aattcacatt caaaagatga agttctggt
 1681 gatgtattta agctttggt caacctggt gccaggccag aaaatgcgca gtatgcttat
 1741 atcgttttc cggaataaa caagccggaa gaaattaaaa aatataatgg aacggcaccg
 1801 aaagtccttg ccaataccaa ccagctgcag gcagttatc atcagcagtt agatatgga
 1861 caggctatct tctatacagc tggaaaatta agcgtagcgg gcatagaaat tgaacagat
 1921 aagccatgtg cagtgtgat caagcacatc aatggcaagc aggtaatttg gctgcccag
 1981 ccattgcaaa aagaaaagac tgcagtgtg agcatcaggg atttaaaaac aggaaaaaca
 2041 aatcgggtta aaattgattt tccgcaacag gaatttcag gtgcaacggt tgaactgaa
 2101 tag

//

SEQ ID NO: 19 Chondriotinase AC nucleic acid deletion NΔ50 C Δ275 (a₂₂₀ – t₁₂₇₈)

atgceatga ccaattggtt gccaaacaac

241 cacctgctac aattggaac tattatacag gcttatattg aaaaagatag tcaactattat
 301 ggcgacgata aagtgttga ccagatttcc aaagctttta agtattggtg tgacagcgac
 361 ccgaaaagcc gcaactggtg gcacatgaa attgccactc cgcaggccct tggatgaatg
 421 ctgactctga tgcgttacgg taaaaagccg ctgatgaag cattggtgca taaattgacc
 481 gaaagaatga agcggggcga accggagaag aaaacggggg ccaacaaaac agatatcgcc
 541 ctgcattact ttatcgtgc ttgttaacg tctgatgagg ctttgettcc ctccgccga
 601 aaagaattgt ttatcccggt acagtttga cactatgagg aaggcctgca atacgattat
 661 tctacactgc agcacggtec gcaattacag atacgagct acggtgccgt atttattacc
 721 ggggtactga aacttgccaa ttacgttagg gataccctt atgctttaag taccgagaaa
 781 ctggtatata ttcaaagta ttaccgcgac agttatctga aagctatccg tggaagttat
 841 atggatttta acgtagaagg ccgaggagta agccggccag acattctaaa taaaaaggca
 901 gaaaaaaga ggttgctggt ggcgaagatg atcgatctta agcactga agaattggct
 961 gatgcgatag ccaggacaga tagcacagtt gcggccggct ataagattga gccctatcac
 1021 catcagttct ggaatggtga ttatgtgcaa catttaagac ctgcctatc ttttaattgt
 1081 cgtatggtga gtaagcggac ccgacgcagt gaatccggca ataaagaaaa cctgctgggc
 1141 aggtatttat ctgatggggc tactaacata caattgcgcg gaccagaata ctataacatt
 1201 atgccgggtat gggaatggga caagattcct ggcataacca gccgtgatta ttaaccgac
 1261 agacctttga cgaagctt

SEQ ID NO: 20 Chondroitinase B nucleic acid Locus CHU27584

ORIGIN

1 atgaagatgc tgaataaact agccggatac ttattgccga tcatggtgct gctgaatgtg
 61 gcacatgct taggtcaggt tgttgcttca aatgaaactt tataccaggt tgtaaaggag

121 gtaaaacccg gtggctcgtt acagattgcc gatgggactt ataaagatgt tcagctgatt
 181 gtcagcaatt caggaaaatc tggttgccc atcactatta aagccctgaa cccgggtaag
 241 gttttttta ccggagatgc taaagtagag ctgaggggag agcacctgat actggaaggc
 301 atctggttta aagacgggaa cagagctatt caggcatgga aatcacatgg acccggtatg
 361 gtggctatat atggtagcta taaccgcatt accgcatgtg ttttgattg tttgatgaa
 421 gccaatctg cttacattac tacttcgctt accgaagacg gaaaggtacc tcaacattgc
 481 cgcatagacc attgcagttt taccgataag atcactttg accaggtaat taacctgaac
 541 aatacagcca gagctattaa agacgggttc gtgggaggac cggggatgta ccatcgtgtt
 601 gatcactgtt tttttccaa tccgcaaaaa ccgggtaatg ccggaggggg aatcaggatt
 661 ggctattacc gtaatgatat aggccgttgt ctggtagact ctaacctgtt tatgcgtcag
 721 gattcggaag cagagatcat caccagcaaa tcgcaggaaa atgtttatta tgtaataact
 781 tacctgaatt gccagggcac catgaacttt cgtcacggtg atcatcaggt ggccattaac
 841 aatttttata taggcaatga ccagegattt ggatacgggg gaatgtttgt ttggggaagc
 901 aggcattgta tagcctgtaa ttatttgag ctgtccgaaa ccataaagtc gagggggaac
 961 gccgcatgtt atttaaacc cgggtctatg gcttcggagc atgctcttc ttcgatatg
 1021 ttgatagcca acaacgctt catcaatgta aatgggtatg ccatccattt taatccattg
 1081 gatgagcgca gaaaagaata ttgtgcagcc aataggctta agttcgaaac cccgcaccag
 1141 ctaatgttaa aaggcaatct tttcttaag gataaacctt atgtttacc atttttaaa
 1201 gatgattatt ttatagcagg gaaaaatagc tggactggtg atgtagcctt aggtgtggaa
 1261 aagggaatcc ctgttaacat ttggccaat aggtctgcct ataagccgtt aaaaattaaa
 1321 gatatccagc ccatagaagg aatcgctctt gatctcaatg cgctgatcag caaaggcatt
 1381 acaggaaagc cccttagctg ggatgaagta aggcctact ggttaaaaga aatgccccgg
 1441 acgtatgctt taacggccag gctttctgca gatagggtc caaagttta agccgtaatt
 1501 aaaagaaata aagagcactg a

SEQ ID NO: 21 Chondriotinase B nucleic acid deletion NΔ120 CΔ120 (a₄₃₆ – g₁₁₇₀)

attac tacttcgctt accgaagacg gaaaggtacc tcaacattgc
 481 cgcatagacc attgcagttt taccgataag atcactttg accaggtaat taacctgaac
 541 aatacagcca gagctattaa agacgggttc gtgggaggac cggggatgta ccatcgtgtt
 601 gatcactgtt tttttccaa tccgcaaaaa ccgggtaatg ccggaggggg aatcaggatt
 661 ggctattacc gtaatgatat aggccgttgt ctggtagact ctaacctgtt tatgcgtcag
 721 gattcggaag cagagatcat caccagcaaa tcgcaggaaa atgtttatta tgtaataact
 781 tacctgaatt gccagggcac catgaacttt cgtcacggtg atcatcaggt ggccattaac
 841 aatttttata taggcaatga ccagegattt ggatacgggg gaatgtttgt ttggggaagc
 901 aggcattgta tagcctgtaa ttatttgag ctgtccgaaa ccataaagtc gagggggaac
 961 gccgcatgtt atttaaacc cgggtctatg gcttcggagc atgctcttc ttcgatatg
 1021 ttgatagcca acaacgctt catcaatgta aatgggtatg ccatccattt taatccattg
 1081 gatgagcgca gaaaagaata ttgtgcagcc aataggctta agttcgaaac cccgcaccag
 1141 ctaatgttaa aaggcaatct tttcttaag

SEQ ID NO: 22 Chondroitinase ABCI nucleic acid Locus I29953

ORIGIN

1 ggaattccat cactcaatca ttaaatttag gcacaacgat gggctatcag cgttatgaca
 61 aatttaatga aggacgcatt ggttcactg ttagccagcg ttctaaagga gaaaaataat
 121 gccgatattt cgtttactg cacttgcaat gacattgggg ctattatcag cgccttataa
 181 cgcgatggca gccaccagca atcctgcatt tgatcctaaa aatctgatgc agtcagaaat
 241 ttaccatttt gcacaaaata acccattagc agacttctca tcagataaaa actcaatact
 301 aacgttatct gataaacgta gcattatggg aaaccaatct cttttatgga aatggaaagg

361 tggtagtagc ttactttac ataaaaaact gattgtcccc accgataaag aagcatctaa
 421 agcatgggga cgctcatcta ccccggttt ctcattttgg cttlacaatg aaaaaccgat
 481 tgatggttat ctactatcg atttcggaga aaaactcatt tcaaccagtg aggctcagge
 541 aggcctttaa gtaaaattag atttcactgg ctggcgtgct gtgggagtct ctttaaataa
 601 cgatcttgaa aatcgagaga tgacctaaa tgcaaccaat acctcctctg atggtaacta
 661 agacagcatt gggcggttct taggtgctaa agtcgatagt attcgttta aagcgcttc
 721 taatgtgagt cagggtgaaa tctatctga ccgtattatg tttctgtcg atgatgctcg
 781 ctaccaatgg tctgattatc aagtaaaaac tcgcttatca gaacctgaaa ttcaattca
 841 caacgtaaag ccacaactac ctgtaacacc tgaaaattta ggggccattg atcttattcg
 901 ccaacgtcta attaataat ttgtcggagg tgaaaaagag acaaacctcg cattagaaga
 961 gaatatcagc aaattaaaaa gtgatttcca tctcttaat attcacact tagcaaatgg
 1021 tggaacgcaa ggcagacatc tgatcactga taaacaaatc attattatc aaccagagaa
 1081 tcttaactcc caagataaac aactatttga taattatgtt attttaggta attacacgac
 1141 attaatgttt aatattagcc gtgcttatgt gctggaaaaa gatccccacac aaaaggcgca
 1201 actaaagcag atgtacttat taatgacaaa gcatttatta gatcaaggct tigttaaagg
 1261 gagtgttita gtgacaaccc atcactgggg atacagtctt cgttggtggt atattccac
 1321 gtattaatg tctgatgcac taaaagaagc gaacctacaa actcaagttt atgattcatt
 1381 actgtggtat tcacgtgagt taaaagtag tttgatatg aaagtaagtg ctgatagtc
 1441 tgatctagat tatttcaata cttatctcg ccaacattta gccttattat tactagagcc
 1501 tgatgatcaa aagcgatca acttagttaa tactttcagc cattatatca ctggcgcat
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 1621 tgaaggcaac tatccgggtt actcttccc agcctttaa aatgcctctc agcttattta
 1681 ttattacgc gataccatc ttacgtggg tgaaagtgg tggaataacc tgaaaaagc
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 1981 tttctatgcc ttaattggcg gtgcttttg tattcatcgt tggcaagata aaatggtgac
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 3481 aatattgtta attaccaaga tgataaaaat acagccacac cactcacttt tatgatgtgg
 3541 atttataatg aaaaacctca atcttcccc ttaacgttag catttaaaca aaataataaa
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 3721 aaccaagccg gaacactctt tttgatcaa atcatcatga gtgtaccgtt agacaatcgt
 3781 tgggcagtag ctgactatca aacaccttac gtaataacg cagtaaacac gatggttagt
 3841 aaaaactgga gtgcattatt gatgtacgat cagatgttc aagccatta cctacttta
 3901 aactcgata ctgaatttcg cgatgaccaa acagaaatgg cttcgattta tcagcgctt
 3961 gaatattatc aaggaattcc

//

SEQ ID NO: 23 TAT fusion chondroitinase ABCI nucleic acid

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 301 aacgttatct gataaacgta gcattatggg aaaccaatct ctttatgga aatggaaagg
 361 tggtagtagc ttactttac ataaaaaact gattgtccc accgataaag aagcatctaa
 421 agcatgggga cgtcatctca ccccgttt ctcattttgg cttacaatg aaaaaccgat
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 541 aggttttaa gtaaaattag atttactgg ctggcgtgct gtgggagtct ctttaaataa
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 2521 aaaaaagata gaaaacatgc cttatcaaac aacacttcaa caaggtgatt ggtaattga
 2581 tagcaatggc aatggttact taattactca agcagaaaaa gtaaatgtaa gtcgccaaca
 2641 tcaggittca gcggaaaata aaaatcgcca accgacagaa ggaaacttta gtcgggcatg
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 3361 ttaccaata cccttaccac ttcaataat aatcaattat cgctaagcaa acagcatgct
 3421 aaagatgggtg aacaatcact caaatggcaa tatcaaccac aagcaacatt aacactaaat
 3481 aatattgtta attaccaaga tgataaaaat acagccacac cactcacttt tatgatgtgg
 3541 atttataatg aaaaacctca atcttcccca ttaacgttag catttaaca aaataataaa
 3601 attgactaa gttttaatgc tgaacttaat ttacgggggt ggcgaggtat tgctgttctt
 3661 tttcgtgata tgcaaggctc tgcgacaggt caacttgatc aattagtgt caccgctcca
 3721 aaccaagccg gaacactctt tttgatcaa atcatcatga gtgtaccgtt agacaatcgt
 3781 tgggcagtac ctgactatca aacaccttac gtaaataacg cagtaaacac gatgggtagt
 3841 aaaaactgga gtgcattatt gatgtacgat cagatgtttc aagcccatta cctacttta
 3901 aactcgata ctgaatttcg cgtgaccaa acagaaatgg ctctgattta tcagcgcttt
 3961 gaatattatc aaggaattcc

SEQ ID NO: 24 An HIV TAT sequence and Gly penta linker protein

G R K K R R Q R R R G G G G

SEQ ID NO: 25 Tat sequence and Gly penta linker nucleic acid

ggg cgt aaa aag cgt cgt caa cgt cgt cgt ggt ggt ggt ggt ggt

SEQ ID NO: 26 Present Invention Chondroitinase ABC II Nucleic acid

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aacacctactgttattaatttagttaagtaa

SEO ID NO: 27 Present Invention Chondroitinase ABC II protein

```
>_ ABC (present invention) 990 aa vs.
>_ ABC (mature) 990 aa
scoring matrix: , gap penalties: -12/-2
98.3% identity; Global alignment score: 6393
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457676	LPTLSHEAFGDIYLFEGELPNIL	TTSNNNQLSLSKQHAKDGEQSLKWQYQPQATLTLNNI				
	:	:	:	:	:	:
—	LPTLSHEAFGDIYLFEGELPNIL	TTSNNNQLSLSKQHAKDGEQSLKWQYQPQATLTLNNI				
	10	20	30	40	50	60
	70	80	90	100	110	120
457676	VNYQDDKNTATPLTFMMWIYNEK	QSSPLTLAFKQNNKIALSFNAELNFTGWRGI	AVPFR			
	:	:	:	:	:	:
—	VNYQDDKNTATPLTFMMWIYNEK	QSSPLTLAFKQNNKIALSFNAELNFTGWRGI	AVPFR			
	70	80	90	100	110	120
	130	140	150	160	170	180
457676	DMQGSATGQLDQLVITAPNQAGTL	FFDQIIMSVPLDNRWAVPDYQTPYVNN	AVNTMVS	SKN		
	:	:	:	:	:	:
—	DMQGSVTGQLDQLVITAPNQAGTL	FFDQIIMSVPLDNRWAVPDYQTPYVNN	AVNTMVS	SKN		
	130	140	150	160	170	180
	190	200	210	220	230	240
457676	WSALLMYDQMFOAHYPTLNFDTE	FRDDQTEMASIYQRF	EYYQGIRSDKKITP	DMLDKHLA		
	:	:	:	:	:	:

12/22

```

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      850      860      870      880      890      900
457676 KAPKYTVLQHNDQLHAVKDKITQEEGYGFFEATKLKSADATLLSSDAPVMVMAKIQNQQ
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      KAPEYTVLQHNDQPHAVKDKITQEEGYAFFEATKLKSADATLLSSDAPVMVMAKIQNQQ
      850      860      870      880      890      900

      910      920      930      940      950      960
457676 TLSIVNPDNLNLYQGREKDQFDDKGNQIEVSVYSRHWLTAESQSTNSTITVKGIWKLTPQ
      .....
      TLSIVNPDNLNLYQGREKDQFDDKGNQIEVSVYSRHWLTAESQSTNSTITVKGIWKLTPQ
      910      920      930      940      950      960

      970      980      990
457676 PGVVIKHHNNNTLITTTTIQATPTVINLVK
      .....
      PGVVIKHHNNNTLITTTTIQATPTVINLVK
      970      980      990

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SEQ ID NO: 28 Present Invention Chondroitinase ABC I nucleic acid

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SEQ ID NO: 29 Present Invention Chondroitinase ABC I protein

365019 ATSNPAFDPKNLMQSEIYHFAQNNPLADFSSDKNSILTSDKRSIMGNQSLWKKWKGSS

.....

365019 FTLHKKLIVPTDKEASKAWGRSSTPVFSFWLYNEKPIDGYLTIDFGEKLISTSEAQAGFK

365019 VKLDFGTWRTVGVSLNNDLENREMTLNATNTSSDGTQDSIGRSLGAKVDSIRFKAPSNVS

365019 QGEIYIDRIMFSVDDARYQWSYQVVKTRLSEPEIQFHNVKPQLPVTENLAAIDLRQRL

365019 INEFVGGEKETNLALEENISKLSDFDALNHTLANGGTQGRHLITDKQIIYQPENLNS

365019 QDKQLFDNYVILGNYTTLMFNISRAYVLEKDPTQKAQLKQMYLLMTKHLLDQGFVKGSAL

365019 VTTHHWGYSSRWYISTLLMSDALKEANLQTQVYDSLWYSREFKSSFDMKVSADSSDL

365019 YFNTLSRQHLALLLLEPDDQKRINLVNTFSHYITGALTQVPPGGKDGLRPDGTAWRHEGN

365019 YPGYSFPAFKNASQLIYLLRDTPFSVGESGWNSLKKAMVSAWIYSNPEVGLPLAGRHLN

365019 SPSLKSVAQGYWLAMSAKSSPKTLASIYLAISDKTQNESTAIFGETITPASLPQGFYA

365019 FNGGAFGIHRWQDKMVTLKAYNTNVWSSEIYNKDNRYGRYQSHGVAQIVSNGSLSQGYQ

365019 QEGWDWNRMPGATTIHLPLKDLDSPKPHTLMQRGERGFSGTSSLEGQYGMMAFDLIYPAN

365019 LERFDPNFTAKKSVAADNHLIFIGSNINSSDKNKNVETTLFQHAIPTLNTLWINGQKI

365019 ENMPYQTTLQQGDWLIDSNGNGYLITQAEKVNVSQRHQVSAENKNRQPTEGNFSSAWIDH

365019 STRPKDASYEYMFVLDATPEKMGEMAQKFRENGLYQVLRKDKDVHIILDKLSNVTGYAF

365019 YQPASIEDKWIKKVNKPAIVMTHRQKDTLIVSAVTPDLNMTRQKAATPVTINVTINGKWQ

365019 SADKNSEVKYQVSGDNTELTFTSYFGIPQEIKLSPLP

hyaluronidase 1 protein, **SEQ ID NO: 30**, Locus HSU96078

hyaluronidase 2 protein, **SEQ ID NO: 31**,

hyaluronidase 3 protein, **SEQ ID NO: 32**, Locus BC012892

hyaluronidase 4 protein, **SEQ ID NO: 33**, AF009010

PH-20 protein, **SEQ ID NO: 34**,

Tat peptide **SEQ ID NO: 35**

ABCI a site-specific mutant designated H501a and Y508a **SEQ ID NO: 36**

SEQ ID NO: 37 Chondroitinase ABCI protein Locus P59807

ORIGIN

1 mpifrtala mtlgllsapy namaatsnpa fdpknlmqse iyhfaqnnpl adfssdknsi
61 ltlsdkrsim gnqslwkwk ggssftlhkk livptdkeas kawgrsstp v fswlynekp
121 idgyltidfg eklstseaq agfkvkldft gwravgvsl n ndlenremtl natntssdgt
181 qdsigrslga kvdsirfkp snvsqgeiyi drimfsvdda ryqwsdyqvk trlsepeiqf

241 hnvkqqlpvt penlaaidli rqrlinefvg geketnlale enisklksdf dalnihtlan
 301 ggtqgrhlit dkqiiyyqpe nlmsqdkqlf dnyvilgnyt tlmfnisray vlekdpqka
 361 qlkqmyllmt khllldqgfvk gsalvtthhw gyssrwwyis tlmsdalke anlqtqvys
 421 llwysrefks sfdmkvsads sldyfnlts rqlhlalle pddqkrinlv ntfshtyga
 481 ltqvppggkd glrpdgtawr hegnypgysf pafknaqli yllrdtpfsv gesgwnnlk
 541 amvsawiysn pevglplagr hpfnsplks vaqgyywlam saksspdktl asiylaisdk
 601 tqnestaifg etitpaslpq gfyafnggaf gihrwqdkmv tlkayntnvw sseiynkdnr
 661 ygryqshgva qivsngsqs qgyqqegwdw nrmegattih lplkdldspk phtlmqrger
 721 gfsstssleg qygmmafni ypanlerfdp nftakksvla adnhlifigs ninssdknkn
 781 vetltfghai tptlntlwin gqkienmpyq tlqqgdwli dsngngylit qaeqvnsrq
 841 hqvsaenknr qptegnfsa widhstrpkd asyeymvfld atpekmgema qkfrenngly
 901 qvlrkdvdh iildklsnvt gyafyqpasi edkwikkvkn paivmthrqk dtlvisavtp
 961 dlnmtrqkaa tptvntvin gkwqsadkns evkyqvsgdn telftsifg ipqeiklsp
 1021 pXX

SEQ ID NO: 38 NA20 ABCI (A₄₅-N₁₀₂₃), protein

aqnnpl adfssdknsi

61 ltldksrism gnqslwkww ggssflhkk livptdkeas kawgrsstpv fsfwlynekp
 121 idgyltidfg eklistseaq agfkvklft gwravgvsl nlenremtl natntssdgt
 181 qdsigrslga kvdsirfkp snvsqgeiyi drimfsvdda ryqwsdyqvk trlsepeiqf
 241 hnvkqqlpvt penlaaidli rqrlinefvg geketnlale enisklksdf dalnihtlan
 301 ggtqgrhlit dkqiiyyqpe nlmsqdkqlf dnyvilgnyt tlmfnisray vlekdpqka
 361 qlkqmyllmt khllldqgfvk gsalvtthhw gyssrwwyis tlmsdalke anlqtqvys
 421 llwysrefks sfdmkvsads sldyfnlts rqlhlalle pddqkrinlv ntfshtyga
 481 ltqvppggkd glrpdgtawr hegnypgysf pafknaqli yllrdtpfsv gesgwnnlk
 541 amvsawiysn pevglplagr hpfnsplks vaqgyywlam saksspdktl asiylaisdk
 601 tqnestaifg etitpaslpq gfyafnggaf gihrwqdkmv tlkayntnvw sseiynkdnr
 661 ygryqshgva qivsngsqs qgyqqegwdw nrmegattih lplkdldspk phtlmqrger
 721 gfsstssleg qygmmafni ypanlerfdp nftakksvla adnhlifigs ninssdknkn
 781 vetltfghai tptlntlwin gqkienmpyq tlqqgdwli dsngngylit qaeqvnsrq
 841 hqvsaenknr qptegnfsa widhstrpkd asyeymvfld atpekmgema qkfrenngly
 901 qvlrkdvdh iildklsnvt gyafyqpasi edkwikkvkn paivmthrqk dtlvisavtp
 961 dlnmtrqkaa tptvntvin gkwqsadkns evkyqvsgdn telftsifg ipqeiklsp
 1021 pXX

SEQ ID NO: 39 NA60 ABCI (F₈₅-N₁₀₂₃) protein

flhkk livptdkeas kawgrsstpv fsfwlynekp

121 idgyltidfg eklistseaq agfkvklft gwravgvsl nlenremtl natntssdgt
 181 qdsigrslga kvdsirfkp snvsqgeiyi drimfsvdda ryqwsdyqvk trlsepeiqf
 241 hnvkqqlpvt penlaaidli rqrlinefvg geketnlale enisklksdf dalnihtlan
 301 ggtqgrhlit dkqiiyyqpe nlmsqdkqlf dnyvilgnyt tlmfnisray vlekdpqka
 361 qlkqmyllmt khllldqgfvk gsalvtthhw gyssrwwyis tlmsdalke anlqtqvys
 421 llwysrefks sfdmkvsads sldyfnlts rqlhlalle pddqkrinlv ntfshtyga
 481 ltqvppggkd glrpdgtawr hegnypgysf pafknaqli yllrdtpfsv gesgwnnlk
 541 amvsawiysn pevglplagr hpfnsplks vaqgyywlam saksspdktl asiylaisdk
 601 tqnestaifg etitpaslpq gfyafnggaf gihrwqdkmv tlkayntnvw sseiynkdnr

661 ygryqshgva qivsngsqsls qgyqqegwdw nrmegattih lplkdldspk phtlmqrger
 721 gfsgtssleg qygmmafnli ypanlerfdp nftakksvla adnhlifigs ninssdknkn
 781 vettlfqhai tptlntlwin gqkienmpyq tlqqgdwli dsngngylit qaekvnvsrq
 841 hqvsanenknr qptegnfsa widhstrpkd asyeymvfld atpekmngema qkfrenngly
 901 qvlrkdldvh iildklsnvt gyafyqpasi edkwikkvkn paivmthrqk dtlivsavtp
 961 dlnmtrqkaa tpvtinvtn gkwqsadkns evkyqvsgdn telftsifg ipqeiklspl
 1021 pXX

SEQ ID NO. 40: NΔ60 CΔ80 ABCI (F₈₅-A₉₄₂) protein

ftlhkk livptdkeas kawgrsstpv fsfwlynekp
 121 idgyltidfg eklistseaq agfkvkl dft gwravgvsl n dlenremtl natntssdgt
 181 qdsigrslga kvdsirfkp snvsqgeiyi drimfsvdda ryqwsdyqvk trlsepeiqf
 241 hnvkpqlpvt penlaaidli rqrlinefv g geketnlale enisklsdf dalnihtlan
 301 ggtqgrhlit dkqiiyyqpe nlnsqdkqlf dnyvilgnyt tlmfnisray vlekdpqka
 361 qlkqmyllmt khildqgf vk gsalvtthw gyssrwwyis tlmsdalke anlqtqvys
 421 llwysrefks sfdmkvsads sldyfntls rqlhlalllle pddqkrinlv ntfshyitga
 481 ltqvppggkd glrpdgtawr hegnypgysf pafknasqli yllrdtpfsv gesgwnnlkk
 541 amvsawiysn pevglplagr hpfnspslks vaqgyywlam saksspdktl asiylaisdk
 601 tqnestaifg etitpaslpq gfyafnggaf gihrwqdkmv tlkayntnvw sseiynkdnr
 661 ygryqshgva qivsngsqsls qgyqqegwdw nrmegattih lplkdldspk phtlmqrger
 721 gfsgtssleg qygmmafnli ypanlerfdp nftakksvla adnhlifigs ninssdknkn
 781 vettlfqhai tptlntlwin gqkienmpyq tlqqgdwli dsngngylit qaekvnvsrq
 841 hqvsanenknr qptegnfsa widhstrpkd asyeymvfld atpekmngema qkfrenngly
 901 qvlrkdldvh iildklsnvt gyafyqpasi edkwikkvkn pa

SEQ ID NO. 41: I. Nucleotide sequence for TAT-ABCI-nΔ20:

1 ggtc gtaaaaagcg tcgtcaacgt cgtcgtctc ctcaatgcgc acaaaataac
 61 ccattagcag acttctcatc agataaaaac tcaatactaa cgttatctga taaacgtagc
 121 attatgggaa accaatctct ttatggaaa tggaaagggtg gtagtagctt tactttacat
 181 aaaaaactga ttgtcccccac cgataaagaa gcatctaaag catggggacg ctcacccacc
 241 cccgttttct cattttggct ttacaatgaa aaaccgattg atggttatct tactatcgat
 301 ttcggagaaa aactcatttc aaccagttag gctcaggcag gctttaaagt aaaattagat
 361 ttactggct ggcgtactgt gggagtctct taaataacg atcttgaaaa tcgagagatg
 421 accttaaatg caaccaatac ctctctgat ggtactcaag acagcattgg gcgttcttta
 481 ggtgctaaag tcgatagtat tcgttttaaa gcgccttcta atgtgagtc gggtgaaatc
 541 tatatcgacc gtattatggt ttctgtcgat gatgctcgct accaatggtc tgattatcaa
 601 gtaaaaactc gcttatcaga acctgaaatt caattcaca acgtaaagcc acaactacct
 661 gtaacacctg aaaatttagc ggccattgat cttatcgcc aacgtctaat taatgaattt
 721 gtcggagggtg aaaaagagac aaacctcgca ttagaagaga atatcagcaa attaaaaagt
 781 gatttcgatg ctcttaatac tcacacttta gcaaatgggtg gaacgcaagg cagacatctg
 841 atactgata aacaaatcat tatttatcaa ccagagaatc ttaactctca agataaacia
 901 ctatttgata attatgttat ttaggtaat tacacgacat taatgtttaa tattagccgt
 961 gcttatgtgc tggaaaaaga tcccacacia aaggcgcaac taaagcagat gtacttatta
 1021 atgacaaaagc atttattaga tcaaggcttt gttaaaggga gtgctttagt gacnaccat
 1081 cactggggat acagttctcg ttggtggtat atttccacgt tattaatgtc tgatgacta
 1141 aaagaagcga acctacaaac tcaagtttat gattcattac tgtggtatc acgtgagttt
 1201 aaaagtagtt ttgatagaa agtaagtgtc gatagctctg atctagatta ttcaataacc

1261 ttatctcgcc aacatttagc ctattacta ctagagcctg atgatcaaaa gcgtatcaac
 1321 ttagttaata ctttcagcca ttatatcact ggcgcattaa cgcaagtgcc accgggtggt
 1381 aaagatgggt tacgccctga tggtagcga tggcgacatg aaggcaacta tccgggctac
 1441 tcttccag ctttaaaaa tgcctctcag ctatttatt tattacgca tacaccattt
 1501 tcagtgggtg aaagtgggtg gaatagcctg aaaaaagcga tggtttcagc gtggatctac
 1561 agtaatccag aagttggatt accgcttgca ggaagacacc ctcttaactc accttcgta
 1621 aaatcagtcg ctcaaggcta ttactggctt gccatgtctg caaatcatc gcctgataaa
 1681 acacttgcac ctattatct tgcgattagt gataaaacac aaaatgaac aactgetatt
 1741 ttggagaaa ctattacacc agcgtcttta cctcaagggt tctatgcctt taatggcggt
 1801 gcttttgta tcatcggtg gcaagataaa atggtgacac tgaaagctta taacaccaat
 1861 gtttggtcat ctgaaattta taacaaagat aaccgttatg gccgttacca aagtcattgt
 1921 gtcgctcaaa tagtgagtaa tggctcgcag ctttcacagg gctatcagca agaaggttgg
 1981 gattggaata gaatgccagg ggcaaccact atccacctc ctcttaaaga cttagacagt
 2041 cctaaacctc atacctaat gcaacgtgga gagcgtggat ttagcggaac atcatccct
 2101 gaaggtcaat atggcatgat ggcattcgat ctatttate ccgccaatct tgagcgttt
 2161 gatcctaatt tcatgcgaa aaagagtgtg ttagccgctg ataactactt aattttatt
 2221 ggtagcaata taaatagtag tgataaaaat aaaaatgttg aaacgacctt attccaacat
 2281 gccattactc caacattaaa tacccttggg attaatggac aaaagataga aaacatgcct
 2341 tatcaaaaca cactcaaca aggtgattgg ttaattgata gcaatggcaa tggttactta
 2401 attactcaag cagaaaaagt aatgtaagt cgccaacatc aggtttcagc ggaaaataaa
 2461 aatcgccaac cgacagaagg aaactttagc tcggcatgga tcgatcacag cactgcccc
 2521 aaagatgcca gttatgagta tatggtctt ttagatgcca cacctgaaaa aatgggagag
 2581 atggcacaaa aattccgtga aaataatggg ttatatcagg ttcttcgtaa ggataaagac
 2641 gttcatatta ttctcgataa actcagcaat gtaacgggat atgccttta tcagccagca
 2701 tcaattgaag acaaatggat caaaaaggtt aataaacctg caattgtgat gactcatcga
 2761 caaaaagaca ctcttattgt cagtgcagtt acacctgatt taaatatgac tcgcaaaaa
 2821 gcagcaactc ctgtcaccat caatgtcacg attaatggca aatggcaatc tgctgataaa
 2881 aatagtgaag tgaatatca ggtttctggt gataaacctg aactgacgtt tacgagttac
 2941 ttggtattc cacaagaaat caactctcg ccactccctt ga

SEQ ID NO. 42: II. Amino acid sequence for TAT-ABCI-n Δ 20:

grkkrrrrppqcaqnpladfssdknsiltlsdkrsimgnqslwkwkggssflhkkli vptdkeaskawgrsstpvfsfw
 lynekp idgyltidfgeklistsea qagfkvkl dftgwr tvgvslndlenrem tlnatntssdgtqdsigrslgakvdsirfkapsnv
 sqgeiyidrimfsvddaryqwsdyqv ktrlsepeiqfhnv kplpvt penlaaidlirqlinefv ggeketnlaleenisklksdfd
 alnthtlanggtqgrhlitdkqiiy qpenlnsqdkqlfdnyvilgnyttlmfnisrayvlek dptqkaqlkqmyllmtkhllldqgv
 kgsalvtthhwgyssrwwyistlmsdalkeanlqtqv ydsllwysrefkssfdmkvsadssdldy fntlsrqlalilllepddqkr
 inlvntfshyitgaltqvppgkdglrpdgtawrh egnypgysfpafknasqliyllrdtpfsvgesgwnslkkamvsawiysnp
 evglplagrhp lnspslksvaqgyywlamsaksspdktlasiylaisdktnestaifgetitpaslpqgfyafnggafgihrwqdk
 mvtilkayntnvwsseiykn dnrygryqshgvaqivsnsgslsqgyqqegwdwnrm pgattihlplklddspkphltmqrger
 gfsgtsslegqygmmafdliyp anlerfdpnftakksvlaadnhlifg sninssdknknvettlfqhaitptlntlwingqkienm
 pyqttlqqgdw lidsngnylitqaekvnvsr qhqvsaenknrqptegnssawidhstrpkdasyeymvfldatpekmgema
 qkfrennglyqvlrkd kdvhil dkl snvtgyafyqpasiedkwikkvnpaivmthr qkdtlivsavtpdlnmtrqkaatpvtin
 vtingkwqsadknsevkyqvsgdnteltf syfgipqeiklsplp

SEQ ID NO. 43: II. Nucleotide sequence for TAT-ABCI-n Δ 60:

ggcgtgtaaaaagcgtcgtcaacgtcgtcgtcctcctcaatgctttactttacataaaaaactgattgtccccaccgataaagaagcatcta
aagcatggggagcgtcatccacccccgtttctcattttggtttacaatgaaaaaccgattgatggttatcttactatcgatttcggagaaa
aactcatttcaaccagtgaggctcaggcaggcgtttaaagtaaaattagatttactggctggcgtactgtgggagtcctttaaataacgat
cttgaatacgtgagagatgaccttaaatgcaaccaatacctcctctgatggtactcaagacagcattggcggttctttaggtgctaaagtcg
atagtattcgtttaaagcgccttctaattgtgagtcagggtgaaatctatcgcaccgtattatgtttctgtcgtatgctcgtaccacgtg
gtctgattatcaagtaaaaactcgtttatcagaacctgaaattcaatttcacaacgtaaagccacaactacgtgaacacctgaaaatttag
cggccattgatcttattcgccaacgtctaattaatgaattgtcggagggtgaaaaagagacaaacctcgcattagaagagaatatcagca
aattaaaaagtgatttcgatgctcttaatactcacacttttagcaaatggtggaacgcaggcgagacatctgatcactgataacaaatcatt
atttatcaaccagagaatcttaactctcaagataaacaactatttgataattatgttatttaggtaattacacgacattaatgtttaatattagc
cgtgcttatgtcgtggaaaaagatcccacacaaaaggcgcaactaaagcagatgacttattaatgacaaagcatttattagatcaaggc
tttgtaaaggagggtgcttttagtgaacnaccatcactggggatacagttctcgttggtgtatatttccacgttattaatgtctgatgactaa
aagaagcgaacctacaaactcaagtttatgattcattactgtggtattcactgagtttaaaagtagtttgatagaaagtaagtgtgata
gtctgtatctagattattcaataccttattctcgccaacatttagccttattactactagagcctgatgatcaaaagcgtatcaacttagttaat
actttcagccattatcactggcgcattaacgcaagtggcaccgggtggttaaagatggtttacgccctgatggtacagcatggcgacat
gaaggcaactatccgggctactcttcccagcctttaaataatgcctctcagcttatttatttacgcgatacaccatttccagtgggtgaa
agtgggttggaatagcctgaaaaaagcgatggtttcagcgtggatctacagtaatccagaagtggattaccgcttcaggaagacaccc
tcttaactcaccttcgttaaaatcagtcgctcaaggctattactggcttgccatgtctgcaaaatcatcgctgataaaacacttgcactatt
tatcttgcgattagtataaaacacaaaatgaatcaactgctattttggagaactattacaccagcgtcttaccctcaaggtttctatgcct
ttaatggcgggtgcttttggtattcatcgttggaagataaaatggtgacactgaaagcttataacaccaatgtttggctatctgaaattataa
caaagataaccggttatggcgttaccaaagtcaggtgtcgtcaaatagttagtaatggctcgcagcttcacagggtatcagcaag
aagggtgggattggaatagaatgccaggggcaaccactatccaccttctcttaaagacttagacagtcctaaacctcataccttaatgc
aacgtggagagcgtggatttagcgggaacatcatccttgaaggtaaatatggcatgatggcattcgatcttattatcccgccaatcttga
gcgttttgatcctaatttactgcgaaaaagagtgtatttagccgtgataatcacttaattttattggtagcaatataaatagtagtataaa
aataaaaatgttgaacgacctatttccaacatgccattactccaacattaaatacccttggattaatggacaaaagatagaaaacatgc
cttatcaaacacacttcaacaagggtgattggttaattgatagcaatggcaatggttacttaattactcaagcagaaaaagtaaatgtaagt
cgccaacatcaggtttcagcggaaaaataaaaatcgccaaccgacagaaggaaactttagctcggcatggatgatcacagcactcgc
cccaagatgccagttatgagtatatggtcttttagatgcgacacctgaaaaaatgggagagatggcacaaaaattccgtgaaaaataat
gggttatatcaggttcttctgaaggataaagacgttcatattattctcgataaaactcagcaatgtaacgggatatgccttttatcagccagca
tcaattgaagacaaatggatcaaaaagggttaataaacctgcaattgtgatgactcatcgacaaaaagacactcttattgtcagtgacgtta
cacctgatttaaatatgactcgcacaaaagcagcaactcctgtcaccatcaatgtcacgattaatggcaaatggcaatcgtctgataaaa
atagtgaagtgaatatcaggtttctggtgataacactgaactgacgtttacgagttactttggtattccacaagaaatcaaacctctcgcca
ctcccttga

SEQ ID NO. 44: IV. Amino acid sequence for TAT-ABCI-nΔ60

grkkrrrrppqcftlhkklivptdkeaskawgrsstpvfsfwlynekpdygidfgeklistseaqagfkvklfdtgwrtvgvsl
nndlenremtlnatntssdgtqdsigrslgakvdsirfkapsnvsggeiyidrimfsvddaryqwsdyqvktlrlsepei qfhvnp
qlpvtpenlaaidlirqlinefvggeketnlaleenisklksdfaldlnthtlanggtqgrhlitdkqiiiyqpenlnsqdkqlfdnyvil
gnyttlmfnisrayvlekdpqkaqlkqmyllmtklhldqgfvkgsalvtthhwgyssrwwyistlmsdalkeanlqtqvysll
wysrefkssfdmkvsadsslddyfntlsrqhlallilepddqkrinlvntfshyitgaltqvppggkdglrpdgtawrhagnypgys
fpafknasqliyllrdtpfsvgesgwslkkmvsauiysnpevgplagrhlpslksvaqgyywlamsaksspdktlasiy
laisdktqnetaifgetitpaslpqgyafnggafgihrwqdkmvtlkayntnvwssseiyknknrygryqshgvaqivsnsgqls
qgyqqegwdwnrmppgattihlplkdlldspkphltmqrgergfsstsslegqygmmafdliypanlerfdpnftakksvlaadn
hlifigninssdknknvettlfqhaitptlntwingqkienmpyqtlqqgdwldsnngylitqaekvnvsrqhqvsenknr
qptegnffssawidhstrpkdasieymvflatpekmgemaqkfrennglyqvlrkdvdhiildklsvntgyafyqpasiedk
wikkvnpaivmthrqkdtlivsavtpdlmnrqkaatpvtinvingkwqsadknsevkyqvsgdnteltftsifgipqeiklsp
lp

SEQ ID NO. 45: V. Nucleotide sequence for ABCI-TAT-C:

gccaccagcaatcctgcatttgatcctaaaaatctgatgcagtcagaaattaccattttgcacaaaataacccattagcagacttctcatc
agataaaaactcaataactaacgttatctgataaacgtagcattatgggaaaccaatctctttatggaaatggaaaggtggtagtagcttta
ctttacataaaaaactgattgtccccaccgataaagaagcatctaaagcatggggacgctcatccacccccgttttctcattttggctttac
aatgaaaaaccgattgatggttacttactatcatttcggagaaaaactcattcaaccagtgaggtcaggcaggctttaagtaaaatt
agatttactggtgctgactgtgggagctctcttaataacgatcttgaaaatcgagagatgacctaaatgcaaccaataacctctctg
atggtactcaagacagcattgggctgttcttaggtgctaaagtcgatagtattcgttttaagcgccttctaattgtgagtcagggtgaaatc
tatatcgaccgtattatgtttctgtcgtatgctcgtaccatggctgtattatcaagtaaaaactcgttatcagaacctgaaattcaatt
tcacaacgtaaaagccacaactacctgtaacacctgaaaatttagcggccattgatcttattcgccaacgtctaattaatgaattgtcggag
gtgaaaaagagacaaacctgcattagaagagaatatcagcaataaaaaagtgatttcgatgctcttaatactcacactttagcaaatg
gtggaacgcaaggcagacatctgactgataaacaatcattattatcaaccagagaatcttaactctcaagataaacaactatttgat
aattatgttttttaggtaattacacgacattaatgtttaattattagccgtgcttatgtgctggaaaaagatcccacacaaaaggcgcaacta
aagcagatgtacttattaatgacaaagcatttattagatcaaggccttgttaaaggagtgctttagtgacnaccatcactggggataca
gttctcgttggtggtatattccacgttattaatgtctgatgcactaaaagaagcgaacctacaaactcaagtttatgattcattactgtggtat
tcacgtgagtttaaaagtagttttgatatgaaagtaagtgtgatgctctgatctagattattcaataaccttatctcgccaacatttagcctt
attactactagacctgatgataaaaagcgtatcaacttagttaatactttcagccattatatactggtcgcattaacgcaagtgccaccgg
gtggtaaagatggtttacgccctgatggtacagcatggcgacatgaaggcaactatccgggctactctttccagccttaaaaaatgcct
ctcagcttatttattattacgcgatacaccattttcagtggtgaaagtgttggaatagcctgaaaaagcgtatggttcagcgtggtatc
acagtaatccagaagttggattaccgcttgaggaaagacaccttctaactcaccttcgttaaaatcagtcgctcaaggctattactggct
tgccatgtctgcaaaatcatcgctgataaaacacttgcatctattatcttgcgattagtataaaacacaaaatgaatcaactgctattttt
ggagaaactattacaccagcgtctttacctcaaggtttctatgccttaattggcgggtgcttttggtattcatcgttggaagataaaatggtg
acactgaaagcttataacaccaatgtttggtcatctgaaattataacaaagataaccgttatggcgttaccaaagtcagtggtcgtcga
aatagttagtaatggctcgcagcttcacagggctatcagcaagaaggttggtggaatagaatgccaggggcaaccactatccac
cttctcttaaaagacttagacagtcctaaacctcataccttaatgcaacgtggagagcgtggtttagcgggaacatcatccctgaaggctc
aatatggcatgatggcattcgtattttatcccgccaatcttgagcgttttgatcctaatttactgcgaaaaagagtgtatttagccgctg
ataatcacttaattttattggtagcaatataaatagtagtgataaaaaataaaatgttgaacgacctattccaacatgccattactccaac
attaaataccctttggattaatggacaaaagatagaaaacatgccttatcaacaacacttcaacaaggtgattggttaattgatagcaatg
gcaatggttacttaattactcaagcagaaaaagtaaatgtaagtcgccaacatcaggttcagcggaaaaataaaaatcgccaaccgaca
gaaggaaactttagctcggcatggatcgtacagcactcgcacaaagatgccagttatgagtatatggtcttttagatgcgacacct
gaaaaaatgggagagatggcacaaaaatccgtgaaaaataatgggttatatcaggttcttcgtaaggataaagacgttcattattctcg
ataaactcagcaatgtaacgggatgcttttatcagccagcatcaattgaagacaaaatggatcaaaaagggttaataaactgcaattgt
gatgactcatcgacaaaaagacactcttattgtcagtgagttacacctgatttaaatatgactcgccaaaaagcagcaactcctgtcacc
atcaatgtcacgattaatggcaaatggcaatctgctgataaaaatagtgaaagtgaatatcaggtttctggtgataacactgaactgacgt
ttacgagttactttggtattccacaagaatcaaaactctcgcactccct
ggctgtaaaaagcgtcgtcaacgtcgtcgtcctcctcaatgctag

SEQ ID NO. 46: V. Amino acid sequence for ABCI-TAT-C:

atsnpafdpknlmqsei yhf aqnnplad fssdknsilt sdrsimgnqsl lwkwkgssfthkkli vptdkeaskawgrsstpv
fsfwlynekp idgy ltidfgek list seaqagfkv kldf gtwrtvgv slnndlenrem tlnatntssdgtqdsigrslgakvdsirfka
psnvsqgei yidrimfsvddaryqwsdyqv ktrlsepeiqfhnv kqp lvp tpenlaaidlirqlnefv ggeketnlaleenisklk
sdfdalnthtlanggtqgrhlitdkqii yqpenlnsqdkqlfdnyvilgn yttlmfnisrayvlekdp tqkaqlkqmyllmtkhlld
qgfvkgsalvtthhwgyssrwwyistllmsdalkeanlqtqv ydsllwysrefkssfdmkvsadssldyfn tlrqhlallllepd
dqkrinlvntfshyitgaltqvppggkdglrpdgtawrh egnypgysfpafknaqliyllr dtpfsvgesgwnslkkamvsawi
ysnpevg lplagrhl npspslksvaqgyywlamsaksspdktlasiylaisdktq nestai fgetitpaslpqgfyafnggafgihr
wqdkmvtlkayntnvwssei ynkdnrygryqshgvaqivsnsgslsqgyqqegwdwnrmpgattihlplkdldspkphltm
qrgergfs gtslegqygmmafdli ypanlerfdpnftaksvlaadnhlifigsninssdknknvettlfqhaitptlntlwingqki

enmpyqtlqqgdwldnsngylitqaekvnvrsqhqvsaenknrqptegnfsawidhstrpkdasymvflidatpekm
 gemaqkfrennglyqlrkdvdhildklsvntgyafyqpasiedkwikkvnkpaivmthrqkdtlivsavtpdlnmtrqkaa
 tpvtinvtingkwqsadknsevkyqvsdnteltftsifgipqeiklsplpgrkrrrppqc

SEQ ID NO. 47 Nucleotide sequence for chondroitinase ABCI-nΔ20

gc acaaaataac
 61 ccattagcag acttctcadc agataaaaaac tcaataactaa cgttatctga taaacgtagc
 121 attatgggaa accaatctct tttatggaaa tggaaagggtg gtagtagctt tactttacat
 181 aaaaaactga ttgtcccccac cgataaagaa gcatctaaag catggggacg ctcacccacc
 241 cccgttttct cattttggtt ttacaatgaa aaaccgattg atggttatct tactatcgat
 301 ttcggagaaa aactcatttc aaccagttag gctcaggcag gctttaaagt aaaattagat
 361 ttcactggct ggcgtactgt gggagtctct ttaaataacg atcttgaaaa tcgagagatg
 421 accttaaatg caaccaatac ctctctgat ggtactcaag acagcattgg gcgttcttta
 481 ggtgctaaag tcgatagtat tcgttttaaa gcgccttcta atgtgagtca gggtgaaatc
 541 tatatcgacc gtattatgtt ttctgtcgat gatgctcgct accaatggct tgattatcaa
 601 gtaaaaactc gcttatcaga acctgaaatt caatttcaca acgtaaagcc acaactacct
 661 gtaacacctg aaaatttagc ggccattgat ctatttcgcc aacgtctaat taatgaattt
 721 gtcggagggtg aaaaagagac aaacctcgca ttagaagaga atatcagcaa attaaaaagt
 781 gatttcgatg ctcttaatac tcacacttta gcaaatgggt gaacgcaagg cagacatctg
 841 atcactgata aacaaatcat tatttatcaa ccagagaatc ttaactctca agataaacia
 901 ctatttgata attatgttat ttaggtaat tacacgacat taatgtttaa tattagccgt
 961 gcttatgtgc tggaaaaaga tcccacacia aaggcgcaac taaagcagat gtacttatta
 1021 atgacaaagc attattaga tcaaggcttt gttaaaggga gtgcttagt gacnaccat
 1081 cactggggat acagttctcg ttgggtggtat atttcacgt tattaatgtc tgatgcacta
 1141 aaagaagcga acctacaaac tcaagtttat gattcattac tgtggtattc acgtgagttt
 1201 aaaagtagtt ttgatatgaa agtaagtgtc gatagctctg atctagatta ttcaatacc
 1261 ttatctgcc aacatttagc ctattacta ctagagcctg atgatcaaaa gcgtatcaac
 1321 ttagttaata ctttcagcca ttatcactt ggcgcattaa cgcaagtgc accgggtggt
 1381 aaagatggtt tacgccctga tggtagcga tggcgacatg aaggcaacta tccgggctac
 1441 tcttccag ctttaaaaaa tgctctcag ctatttatt tattacgga tacaccattt
 1501 tcagtgggtg aaagtgggtg gaatagcctg aaaaaagcga tggtttcagc gtggatctac
 1561 agtaatccag aagtggatt accgcttga ggaagacacc ctcttaactc acctcgta
 1621 aaatcagtcg ctcaaggcta ttactggctt gccatgtctg caaatcadc gectgataaa
 1681 acacttgcct ctatttatct tgcgattagt gataaaacac aaatgaatc aactgctatt
 1741 ttggagaaa ctattacacc agcgtcttta cctcaagggt tctatgcctt taatggcggt
 1801 gcttttggtt tcatcggtg gcaagataaa atggtagcac tgaaagetta taacaccaat
 1861 gtttggtcat ctgaaattta taacaaagat aaccgttatg gccgttacca aagtcaggt
 1921 gtcgtcaaaa tagtgagtaa tggctcgag ctttcacagg gctatcagca agaagggttg
 1981 gattggaata gaatgccagg ggcaaccact atccacctc ctctaaaga ctagacagt
 2041 cctaaacctc atacctaat gcaacgtgga gagcgtggat ttagcggaac atcatccctt
 2101 gaaggtcaat atggcatgat ggcattcgat ctatttate ccgcaatct tgagcggtt
 2161 gatcctaatt tcatcgcaa aaagagtgtt ttagccgtg ataactactt aattttatt
 2221 ggtagcaata taaatagtag tgataaaat aaaaatgtt aaacgacctt attccaacat
 2281 gccattactc caacattaaa tacccttgg attaatggac aaaagataga aaacatgcct
 2341 tatcaaaacia cacttcaaca aggtgattgg ttaattgata gcaatggcaa tggttactta
 2401 attactcaag cagaaaaagt aaatgtaagt cgccaacatc aggtttcagc ggaaaaataa
 2461 aatcgccaac cgacagaagg aaacttagc tcggcatgga tcgatcacag cactcgcccc
 2521 aaagatgcca gttagagta tatggtcttt ttagatgca cacctgaaaa aatgggagag
 2581 atggcacaaa aattccgtga aaataatggg ttatcagag ttctctgtaa ggataaagac

2641 gtccatatta ttctgataa actcagcaat gtaacgggat atgccttta tcagccagca
 2701 tcaattgaag acaaatggat caaaaagggt aataaacctg caattgtgat gactcatcga
 2761 caaaaagaca ctcttattgt cagtgcagtt acacctgatt taaatatgac tcgccaaaaa
 2821 gcagcaactc ctgtcccat caatgtcacg attaattggca aatggcaatc tgctgataaa
 2881 aatagtgaag tgaatatca ggttctggt gataaacctg aactgacgtt tacgagttac
 2941 ttggtattc cacaagaaat caaactctc cactccctt ga

SEQ ID NO. 48: II. Nucleotide sequence for ABCI-nΔ60:

ttactttacataaaaaactgattgtccccaccgataaagaagcatctaaagcatggggacgctcatccacccccgtttctcattttggcctt
 tacaatgaaaaaccgattgatgggtattcttactatcgatttcggagaaaaactcatttcaaccagtgaggctcaggcaggcctttaaagtaa
 aattagatttcactggctggcgtactgtgggagctctttaaataacgatcttgaatacgcagagatgaccttaaatgcaaccaatacctcc
 tctgatggactcaagacagcattggcgttctttaggtgctaaagtcgatagatttcgtttaaagcgcccttctaattgtgagtcagggtga
 aatctatcgcagcgtattatgtttctgtcgtatgctcgtaccaatggctgtgattatcaagtaaaaactcgcttatcagaacctgaaatt
 caattcacaacgtaaagccacaactacctgtaacacctgaaaatttagcgccattgatcttattcgccaacgtctaattaatgaattgtc
 ggaggtgaaaaagagacaaacctgcattagaagagaatatcagcaaaataaaagtatttcgatgctcttaatactcacactttagca
 aatgggtggaacgcaaggcagacatctgatcactgataaacaatcattattatcaaccagagaatcttaactctcaagataaacaactat
 ttgataattatgttattttaggtaattacacgacattaatgttataattagcgctgcttattgtgctgaaaaagatccacacaaaaggcgca
 actaaagcagatgtacttattaatgacaaagcatttattagatcaaggcttgttaaaggagtgctttagtgacnaccatcactggggat
 acagttctcgttgggtgatttccacgttattaatgtctgatgcactaaaagaagcgaacctacaaactcaagtttatgattcattactgtg
 gtattcacgtgagtttaaaagtagtttgatatgaaagtaagtgtctgatctagattatttcaataccttatctcgccaacatttag
 ccttattactactagacgtgatgataaaagcgtatcaacttagttaactttcagccattatcactggcgcaattaacgcaagtgccac
 cgggtggtaaagatgggttacgcccgtgatgttacagcatggcgacatgaaggcaactatccgggctactcttcccagcctttaaataat
 gcctctcagcttatttatttattacgcgatacaccattttcagtggtgaaagtgggtggaatagcctgaaaaagcgatgggttcagcgtg
 gatctacagtaatccagaagtggattaccgcttgcagggaagacacctcttaactcaccttcgttaaaatcagtcgctcaaggctattact
 ggcttgcctgtctgcaaaatcatcgcctgataaaacacttgcctctatttattcttgcgattagtataaaacacaaaatgaatcaactgcta
 ttttggagaaactattacaccagcgtcttacctcaagggttctatgcctttaaagtgcggtgcttttggtattcatcgttggcaagataaaatg
 gtgacactgaaagcttataacaccaatgttgggtcatctgaaattataacaagataaccgttatggcgttaccaaagtcagtggtgctgc
 tcaaatagtgagtaattggctcgcagcttccacagggctatcagcaagaaggttgggattggaatagaatgccaggggcaaccactatc
 caccttctctttaaagacttagacagtcctaaacctcataccttaagtgaacgtggagagcgtggatttagcggaacatcatcccttgaag
 gtcaatatggcatgatggcattcgatcttattatcccgcgaatcttgagcgttttgatcctaatttactgcgaaaaagagtgatttagccg
 ctgataactacttaattttattggtagcaatataaatagtagtgataaaaataaaaatgttgaaacgaccttattccaacatgccattactcc
 aacattaaatacccttggattaatggacaaaagatagaaaacatgccttatcaacaacacttcaacaaggtgattggttaattgatagc
 aatggcaatggttacttaattactcaagcagaaaaagtaaatgtaagtcgccaacatcaggttccagcggaataaaaaatcgccaacc
 gacagaaggaaactttagctcggcatggatcgatcacagcactcgcacaaagatgccagttatgagtatatggtcttttagatgcgac
 acctgaaaaaatgggagagatggcacaaaaattccgtgaaaataatgggttatatcaggttcttcgtaaggataaagacgttcatattatt
 ctgataaaactcagcaatgtaacgggatatgccttttatcagccagcatcaattgaagacaaatggatcaaaaagggttaataaacctgca
 attgtgatgactcatcgacaaaaagacactcttattgtcagtgacgttacactgtattaaatgactcgccaaaaagcagcaactcctgt
 caccatcaatgtcacgattaatggcaatggcaatctgctgataaaaatagtgaaagtgaatatcaggttctggtgataacactgaactg
 acgtttacgagttacttgggtattccacaagaaatcaactctcgccactccctga

SEQ ID NO. 49: Nucleotide sequence for TAT

ggtcgtaaaaagcgtcgtcaacgtcgtcgtcctcctcaatgc

(SEQ ID NO. 50) Amino acid sequence for a TAT peptide

grkkrrqrrrppqc

